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(54) Title: HIGH MOBILITY GROUP BOX I MUTANT

(57) Abstract: This disclosure relates to modified viruses, e.g., oncolytic vaccinia viruses, which have been modified to contain an exogenous nucleic acid that codes for a variant HMGB1 protein. It provides, at least in part, that vaccinia viruses modified to contain nucleic acid encoding variant HMGB1 and that express a variant HMGB1 or a fragment thereof can achieve a synergistic effect in combination with chemotherapy. Further, this disclosure provides for oncolytic vaccinia viruses and methods of using them in the treatment of various cancers.

HIGH MOBILITY GROUP BOX I MUTANT

GRANT INFORMATION

[0001] This disclosure was made with government support under CA140215 awarded by the National Institutes of Health. The government has certain rights in the disclosure.

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.

CROSS-REFERENCE

[0003] This application claims the benefit of U.S. Provisional Application No 62/397,523 filed September 21, 2016, which is incorporated by reference herein in its entirety.

SUMMARY

[0004] One embodiment provides an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 13, or a fragment thereof. In some embodiments, the exogenous nucleic acid that codes for the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 13, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15. In some embodiments, the oncolytic vaccinia virus the

exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 15.

[0005] One embodiment provides an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises a mutation in its first (NLS1), the second nuclear localization signal (NLS2), or any combinations thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 further comprises a mutation in boxA, boxB, or any combinations thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the HMGB1 variant comprises cysteine residues at positions 43 and 65 of SEQ ID NO: 15. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the HMGB1 variant comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein said HMGB1 variant comprises residues 21-74, and 109-128 or 170-203 of SEQ ID NO: 15. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 15. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid sequence that codes for the variant HMGB1, wherein said nucleic acid sequence is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% to SEQ ID NO: 13, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for an antibody or an antigen binding fragment thereof operably linked to the exogenous nucleic acid sequence that codes for the variant HMGB1. In some embodiments, the oncolytic vaccinia virus further comprising an exogenous nucleic acid sequence that codes for a hyaluronidase operably linked to the exogenous nucleic acid sequence that codes for the variant HMGB1. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes

for the variant HMGB1 comprising the mutation in NLS1, wherein the mutation in NLS1 promotes cytoplasmic re-location of the variant HMGB1. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutation in NLS2, wherein the mutation in NLS2 promotes cytoplasmic re-location of the variant HMGB1. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutations in NLS1 and NLS2, wherein the mutations in NLS1 and NLS2 promote cytoplasmic re-location of the variant HMGB1.

[0006] One embodiment provides an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 is less than or equal to 95% homologous to human HMGB1 (SEQ ID NO: 16). In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 1-85, 89-108, and 150-183 of SEQ ID NO: 16. In some embodiments, the variant HMGB1 comprises residues 1-85 of SEQ ID NO: 16. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 89-108 of SEQ ID NO: 16. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 150-183 of SEQ ID NO: 16. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 1-85, 89-108, and 150-183 of SEQ ID NO: 16. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1wherein the variant HMGB1 comprises mutations at one or more of residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1wherein the variant HMGB1 comprises mutations at residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

[0007] One embodiment provides an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises residues 23, 45, 89-108, and 150-183 of SEQ ID NO: 16, and wherein the variant HMGB1 comprises an amino acid sequence that is less than or equal to 95% homologous to SEQ ID NO: 16. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 23-45, 89-108, 150-183 of SEQ ID NO: 16. In some embodiments, the variant HMGB1 comprises mutations at one or more of residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16. In some

embodiments, the variant HMGB1 comprises mutations at residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

[0008] One embodiment provides an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 14, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 14, or the fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 17, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 17.

[0009] One embodiment provides an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 19, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 14, or the fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 17, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus comprising the exogenous

nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 17. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutation in NLS2, wherein the mutation in NLS2 promotes cytoplasmic re-location of the variant HMGB1. In some embodiments, the oncolytic vaccinia virus comprising the mutations in NLS1 and NLS2, wherein the mutations in NLS1 and NLS2 promotes cytoplasmic re-location of the variant HMGB1. In some embodiments, the variant HMGB1 expressed from the vaccinia virus is secreted into the cytoplasm. In some embodiments, expression of the variant HMGB1 from the vaccinia virus enhances cellular autophagy, compared to an otherwise identical vaccinia virus that lacks the exogenous nucleic acid coding for the variant HMGB1. In some embodiments, expression of the variant HMGB1 enhances tumor-specific replication of the vaccinia virus, compared to an otherwise identical vaccinia virus that lacks the exogenous nucleic acid coding for the variant HMGB1. In some embodiments, expression of the variant HMGB1 enhances cytotoxic immune response against a tumor cell infected by the vaccinia virus, compared to an otherwise identical vaccinia virus that lacks the exogenous nucleic acid coding for the variant HMGB1. In some embodiments, expression of the variant HMGB1 induces cytotoxic immune response against a tumor cell in the vicinity of a tumor cell infected by the vaccinia virus, wherein the tumor cell in the vicinity is not infected by the vaccinia virus. In some embodiments, the vaccinia virus comprises one or more deletions in its genome. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the vaccinia virus comprises a thymidine kinase gene deletion and the exogenous nucleic acid is inserted into the thymidine kinase gene locus. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the vaccinia virus comprises deletions of one or more virulence genes. In some embodiments, the oncolytic vaccinia virus is an extracellular enveloped virus (EEV). In some embodiments, the oncolytic vaccinia virus further expresses a hyaluronidase enzyme. In some embodiments, vaccinia virus expresses the hyaluronidase enzyme, and wherein the hyaluronidase enzyme increases the spread of the vaccinia virus in a tumor microenvironment, compared to the spread of an otherwise identical vaccinia virus that does not comprise the

exogenous nucleic acid coding for the hyaluronidase enzyme operable linked to the exogenous nucleic acid coding for the variant HMGB1. In some embodiments, the vaccinia virus expresses the hyaluronidase enzyme, and wherein the hyaluronidase enzyme increases the spread of the vaccinia virus in a tumor microenvironment. In some embodiments, the oncolytic vaccinia virus the vaccinia virus further expresses a protein that stabilizes the variant HMGB1 in circulation. In some embodiments, the protein that stabilizes the variant HMGB1 in circulation is an immunoglobulin or a domain thereof. In some embodiments, the oncolytic vaccinia virus the immunoglobulin is IgE. In some embodiments, the immunoglobulin domain is an Fc domain. In some embodiments, the oncolytic vaccinia virus comprising the exogenous nucleic acid sequence that codes for the variant HMGB1, wherein the further exogenous nucleic acid sequence coding for the IgE is fused upstream to the variant HMGB1 coding sequence. In some embodiments, the oncolytic vaccinia virus further expresses an efflux pump blocker. In some embodiments, the oncolytic vaccinia virus further expresses a chemotherapy sensitizer. In some embodiments, the oncolytic vaccinia virus the virus comprises a modification that enhances sensitization to chemotherapy. In some embodiments, the oncolytic vaccinia virus comprises a modification that attracts chimeric antigen receptor T cells. In some embodiments, the oncolytic vaccinia virus enhances efficacy of a CAR T cell based therapy.

[0010] One embodiment provides an isolated polynucleotide that codes for a variant HMGB1, wherein the polynucleotide comprises a sequence that is at least 85% homologous to SEQ ID NO: 13, or a fragment thereof. In some embodiments, the polynucleotide codes for a variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof. In some embodiments, the oncolytic vaccinia virus the variant HMGB1, wherein the variant HMGB1 comprises a mutation in its first (NLS1) or second nuclear localization signal (NLS2). In some embodiments, the variant HMGB1 further comprises a mutation in boxA or boxB. In some embodiments, the oncolytic vaccinia virus the variant HMGB1 further comprises mutations in boxA or boxB. In some embodiments, the variant HMGB1 comprises cysteine residues at positions 43 and 65 of SEQ ID NO: 15. In some embodiments, the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-183 of SEQ ID NO: 15. In some embodiments, said HMGB1 variant comprises residues 21-105, 108-182, and 183-205 of SEQ ID NO: 15.

[0011] One embodiment provides a pharmaceutical composition comprising an oncolytic vaccinia virus as described in this disclosure or an isolated polynucleotide as described in this

disclosure, a solubilizing agent, and an excipient. In some embodiments, 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 combination thereof. In some embodiments, the excipient comprises di-sodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, myo-inositol, sorbitol, or any combination thereof. In some embodiments, the pharmaceutical composition does not comprise a preservative. In some embodiments, the pharmaceutical composition further comprises one or more of a preservative, a diluent, and a carrier. In some embodiments, the pharmaceutical composition further comprises an additional active ingredient or a salt thereof. In some embodiments, the solubilizing agent is sterile water. In some embodiments, the pharmaceutical composition further comprises an additional active ingredient, wherein the additional active ingredient is a further oncolytic virus.

[0012] One embodiment provides a process for producing an oncolytic vaccinia virus as described in any one of claims 1-67, the process comprising: (i) generating a modified vaccinia virus DNA vector by operably linking a vaccinia virus base nucleic acid sequence to the exogenous nucleic acid sequence in this disclosure; (ii) transfecting mammalian cells with the modified vaccinia virus DNA vector; (iii) culturing the mammalian cells in 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. In some embodiments, the exogenous nucleic acid, as described in this disclosure, in the modified vaccinia virus DNA vector promotes a population of viral particles predominantly containing extracellular enveloped viruses (EEV). In some embodiments, the exogenous nucleic acid, as described in this disclosure, in the modified vaccinia virus DNA vector results in a higher titer in at least one of HeLa cells and 293 cells, compared to an otherwise identical vaccinia virus DNA vector which does not comprise the exogenous nucleic acid according to this discloure.

[0013] One embodiment provides a method of treating a cancer, comprising administering to a subject a therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition according to this disclosure. In some embodiments, the method comprises administration of the therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition as described in this disclosure, wherein the cancer is a solid tumor, a leukemia, or a lymphoma. One embodiment provides a method of treating a tumor, comprising administering to a subject a therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition as described in this disclosure. In some embodiments, the method

comprises administration of the therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition as described in this disclosure, wherein the tumor is a solid tumor, a leukemia, or a lymphoma. In some embodiments, the method comprises administering to the subject in need thereof the a therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition as described in this disclosure.

[0014] One embodiment provides a method of treating a tumor comprising high bioavailability of fatty acids, comprising administering an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition as described in this disclosure, wherein the vaccinia virus demonstrates increased tumor selective replication in the tumor comprising the high bioavailability of free fatty acids. One embodiment provides a method of treating an obese cancer patient, comprising administering an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition according to as described in this disclosure, wherein the vaccinia virus demonstrates increased tumor selective replication in the obese cancer patient. In some embodiments, the increased tumor selective replication is relative to a tumor selective replication of an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid sequence coding for the variant HMGB1. In some embodiments, the increased tumor selective replication is relative to the tumor selective replication of the vaccinia virus in a non-obese cancer patient. In some embodiments, the vaccinia virus produces an increased proportion of EEV in the obese cancer patient. In some embodiments, the increased proportion of EEV in the obese cancer patient is relative to the proportion of EEV produced by an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid sequence coding for the variant HMGB1. In some embodiments, the increased proportion of EEV in the obese cancer patient is relative to the proportion of EEV produced by the vaccinia virus in a non-obese cancer patient.

[0015] One embodiment provides a method of reducing a likelihood of cancer relapse after chemotherapy, comprising administering an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical formulation as described in this disclosure.

[0016] One embodiment provides a method of treatment, comprising administering an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical formulation as described in this disclosure to a subject who has failed a prior treatment comprising an immunotherapy.

[0017] One embodiment provides a method of treatment, comprising administering an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical formulation as described in this disclosure to a subject who has relapse of cancer after chemotherapy.

[0018] One embodiment provides a method of treatment, comprising administering an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical formulation as described in

this disclosure to a subject who has a peritoneal cancer. In some embodiments, the subject is obese. In some embodiments, the method comprises administration of an oncolytic vaccinia virus as described in any one of claims 1-67, or a pharmaceutical formulation as described in any one of claims 76-83, wherein the method further comprises administration of a further therapy. In some embodiments, the method comprises administration of the further therapy in combination with an oncolytic vaccinia virus as described in any one of claims 1-67 or a pharmaceutical formulation as described in any one of claims 76-83, 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. In some embodiments, the further therapy comprises chemotherapy. In some embodiments, the combination of administration of an oncolytic vaccinia virus as described herein or a pharmaceutical formulation as described herein, and chemotherapy, results in a synergistic effect, compared to a combination of the chemotherapy and an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid that codes for a variant HMGB1 as described herein. In some embodiments, the chemotherapy in combination with an oncolytic vaccinia virus as described in any one of claims 1-67 or a pharmaceutical formulation as described herein is administered at a lower dose than a chemotherapy in combination with an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid that codes for a variant HMGB1 as described herein. In some embodiments, the method comprises administration of the further therapy, wherein the further therapy is administered concurrently or sequentially. In some embodiments, the method comprises sequential administration of the further therapy, wherein the further therapy is administered prior to administering an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical composition as described in this disclosure. In some embodiments, the method comprises sequential administration of the further therapy, wherein the further therapy is administered after administering an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical composition as described in this disclosure. One embodiment provides a method of producing a toxic effect in cancer cells, the method comprising administering to a population of cancer cells a therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical composition as described in this disclosure. In some embodiments, the method comprises the administration of the therapeutically effective amount of an oncolytic vaccinia virus as described in this disclosure, or a pharmaceutical composition as described in this disclosure, wherein not every cancer cell in the population of cancer cells is infected with the oncolytic vaccinia virus. In some embodiments, the growth of a non-infected cancer cell is inhibited without direct infection of the oncolytic vaccinia virus. In some embodiments, the

cytotoxic immune response in a non-infected cancer cell is induced without direct infection by the oncolytic vaccinia virus. One embodiment provides a method for drug delivery comprising introducing into a tumor of a subject an oncolytic vaccinia vector as described in this disclosure, or a pharmaceutical formulation as described in this disclosure. In some embodiments, the drug comprises a nanoparticle conjugated to a tumor receptor ligand. One embodiment provides a method for treating cancer in a subject, comprising administering cells infected with an oncolytic vaccinia virus as described in this disclosure. In some embodiments, the method increases efficacy of oncolytic vaccinia virus based cancer therapy. In some embodiments, the method comprises administration of an oncolytic vaccinia virus as described in this disclosure or a pharmaceutical composition as described in this disclosure, wherein 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. In some embodiments, the method comprises administration of an oncolytic vaccinia virus according to this disclosure or a pharmaceutical composition as described in this disclosure, wherein 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. In some embodiments, the method comprises the administration of the oncolytic vaccinia virus according to this disclosure or a pharmaceutical composition as described in this disclosure, wherein 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. In some embodiments, the method comprises 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. In some embodiments, the first, second, and third periods of time are each from about 1 week to about 3 weeks. In some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein 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. In some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein the oncolytic vaccinia virus or the pharmaceutical

composition is administered in a liquid dosage form, a solid dosage form, 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 some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein 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. In some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein 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. In some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein the oncolytic vaccinia virus or the pharmaceutical composition is administered intravenously, intraperitoneally, or by an intratumoral injection. In some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein the vaccinia virus or the pharmaceutical formulation is administered as a bolus injection or a slow infusion. In some embodiments, the method comprises administering the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure, wherein the administration of 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. In some embodiments, the method comprises 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. In some embodiments, the method comprises 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. In some embodiments, the method comprises 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, in a liposomal formulation, a dosage form comprising nanoparticles, a dosage form comprising microparticles, a polymeric dosage form, or any combinations thereof. In some embodiments, the method comprises administration of the further therapy, wherein the further therapy is administered orally, intravenously, by an intratumoral injection, or by

radiation. In some embodiments, the method comprises administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure to a subject in need thereof, wherein the subject is human. In some embodiments, the method comprises administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure to the subject in need thereof, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition the subject has been diagnosed with a cancer or a tumor. In some embodiments, the method comprises the administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure to the subject in need thereof, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition the subject is diagnosed with a cancer or a tumor, and wherein the subject is obese. In some embodiments, the method comprises administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure to the subject in need thereof in combination with the further therapy, wherein prior to administration of 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 comprises administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure to the subject in need thereof in combination with the further therapy, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition the subject is diagnosed with a cancer or a tumor, and wherein the subject is obese. In some embodiments, the subject is administered a ketogenic diet prior to, concurrently, or following administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure. In some embodiments, the subject is on short term fasting prior to, concurrently, or following administration of the oncolytic vaccinia virus as described in this disclosure, or the pharmaceutical composition as described in this disclosure.

[0019] One embodiment provides a modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 13, or a fragment thereof. One embodiment provides a modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises a mutation in its first nuclear localization signal (NLS1), second nuclear localization signal (NLS2), or any combinations thereof. One embodiment provides a modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 is less than or equal to 95% homologous to human HMGB1 (SEQ ID NO: 16). One embodiment provides a modified

virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 23, 45, 89-108, and 150-183 of SEQ ID NO: 16, and wherein the variant HMGB1 comprises an amino acid sequence that is less than or equal to 95% homologous to SEQ ID NO: 16. One embodiment provides a modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 14, or a fragment thereof. One embodiment provides a modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 19, or a fragment thereof. In some embodiments, the modified virus comprises Herpes Simplex Virus (HSV), Adenovirus, Polio virus, VSV, Coxsackievirus, Reovirus, Lentivirus, Adeno-associated virus (AAV), Measles virus, Maraba virus, Newcastle disease (NDV), Seneca valley virus, Mengovirus, or Myxomavir.

[0020] In some embodiments, the oncolytic vaccinia virus comprises a NLS1 and a NLS2, wherein NLS1 comprises SEQ ID NO: 20 and NLS2 comprise SEQ ID NO: 21.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] 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.

[0001] **FIGURE 1** shows the structure and expression of an exemplary variant HMGB1 according to this disclosure. Figure 1 (a) is an illustration of an exemplary variant HMGB1 protein (mHMGB1mut) with mutations in the first nuclear localization signal (NLS1) region located in Box A, and in the second nuclear localization signal (NLS2) region between Box B and the acid C-tail; and an IgE sequence attached to the 5' end of the protein, as compared to a HMGB1 wildtype protein (mHMGB1 wt). Figure 1 (b) is an illustration of the genome of a vaccinia virus comprising a coding sequence for the variant HMGB1 protein (mHMGB1mut). Figure 1 (c) is an image of a gel electrophoresis showing the difference in molecular weight between HMGB1 wildtype and HMGB1mut and demonstrating expression of HMGB1mut from a strain of vaccinia virus. Figure 1 (d) is a graph showing the amount of HMGB protein secreted after infection of the indicated cells (Renca and 4T1) with a negative control, TK (control oncolytic vaccinia virus with a deletion in the viral thymidine kinase gene) or TK-HMGB1mut (oncolytic vaccinia virus with a deletion in the viral thymine kinase gene and insertion of a coding sequence for expression of the exemplary variant HMGB1, HMGB1mut).

[0002] **FIGURE 2** shows effect of treatment with vaccinia virus containing the exemplary variant HMGB1 (HMGB1mut) on tumor growth. Figures 2 (a) and (b) are graphs showing the relative tumor size in mice bearing Renca or MC38 tumors, after being injected with PBS, TK (control oncolytic vaccinia virus with a deletion in the viral thymidine kinase gene), or TK-HMGB1mut (oncolytic vaccinia virus with a deletion in the viral thymine kinase gene and insertion of a coding sequence for expression of the exemplary variant HMGB1, HMGB1mut).

[0003] **FIGURE 3** shows the effect of treatment with vaccinia virus containing the exemplary variant HMGB1 (HMGB1mut) on the cellular immune response in four different cell lines, including Renca, MC38, 4T1, and mouse embryonic fibroblasts (MEFs), and on tumor growth in mice. Shown are levels of (a) phospho-inhibitor of nuclear factor kappa-B kinase subunit beta (pIKK -6); (c) interferon gamma-induced protein 10 (IP-10); (d) tumor necrosis factor alpha (TNF-□); and (e) interferon beta (IFN-□) in cell lines treated with control (PBS), TK (control oncolytic vaccinia virus with a deletion in the viral thymidine kinase gene), or TK-HMGB1mut (oncolytic vaccinia virus with a deletion in the viral thymine kinase gene and insertion of a coding sequence for expression of the exemplary variant HMGB1, HMGB1mut). Figure 3 (f) is a graph showing the cellular immune response to vaccinia virus evaluated by IFN-□ ELISpot assay in mice bearing tumors and treated with PBS, TK, or TK-HMGB1mut. Figure 3 (g) is a graph showing the relative tumor size in mice bearing implanted tumors after treatment with PBS, TK, or TK-HMGB1.

[0004] **FIGURE 4** shows the effect of treatment with vaccinia virus containing the exemplary variant HMGB1 (HMGB1mut) on viral replication and tumor cell survival rate in cell lines and tumor-bearing mice. Figure 4 (a) contains graphs showing increased viral replication in Renca, MC38, and 4T1 cells with TK-HMGB1mut expression compared with TK expression from vaccinia virus. Figure 4 (b) includes graphs illustrating the survival rate of Renca and MC38 cells following treatment with TK or TK-HMGB1mut. Figure 4 (c) shows viral gene expression in mice bearing Renca cell-derived tumors treated with TK or TK-HMGB1mut. Figure 4 (d) contains graphs illustrating the survival rate of 4T1 cells and MEFs following treatment with TK or TK-HMGB1mut. Figure 4 (e) shows viral gene expression in mice bearing MC38 cell-derived tumors treated with TK or TK-HMGB1mut. Figure 4 (f) illustrates viral replication *in vivo* in the whole body and (g) in the tumor of an immune-deficient mouse treated with TK or TK-HMGB1mut.

[0005] **FIGURE 5** shows the effect of treatment with vaccinia virus containing the exemplary variant HMGB1 (HMGB1mut) on autophagy and viral replication in cell lines. Light chain 3 (LC3) is a soluble protein used as a marker of autophagy. Figure 5 (a) is a graph showing the percentage of LC3+ cells in a cell line treated with PBS, TK, or TK-HMGB1mut. Figure 5 (b)

contains graphs showing viral replication in Renca, MC38, and 4T1 cell lines treated with TK or TK-HMGB1mut alone or in combination with inhibitors of autophagic vacuole maturation, including bafilomycin A or LY294002.

[0006] **FIGURE 6** shows the effects of Atg5 knockdown and fatty acids on viral replication in cell lines and tumor-bearing mice. Figure 6 (a) is a graph showing viral replication as a function of time in HCT 116 cells transfected without or with siRNA targeting Atg5. Figure 6 (b) is a graph showing viral replication as a function of time in MDA-MB-231 cells treated without or with cerulenin, a fatty acid inhibitor. Figure 6 (c) are bioluminescent images showing viral gene expression from tumors implanted into the peritoneal cavity for TK or TK-HMGB1mut expression in mice fed a control diet or a high-fat diet. Figure 6 (d) is a graph quantifying viral gene expression from tumors implanted into the peritoneal cavity for TK or TK-HMGB1mut expression at day 3 post treatment in mice fed a control diet or a high-fat diet (HFD).

[0007] **FIGURE 7** shows the effects of PBS, TK, or TK-HMGB1mut alone or in combination with the chemotherapeutic agent paclitaxel (Taxol®) on tumor cell survival rate and tumor growth. Figure 7 (a) is a graph showing the survival rate of tumor cells after exposure to paclitaxel after being fed media taken from UCI-101 cells treated with PBS, TK, or TK-HMGB1mut. Figure 7 (b) shows relative tumor size as a function of time in mice treated with the indicated combinations of paclitaxel, TK, and TK-HMGB1mut.

[0008] **FIGURE 8** shows the effect of a vaccinia strain alone or in combination with matrix metalloproteinase-8 (MMP8) expression or expression of the hyaluronidase PH2O. Figure 8 (a) contains bioluminescent images representing viral gene expression from tumors in mice treated with a vaccinia strain (WR) or strains also expressing MMP8 or PH2O. Figure 8 (b) shows quantification of bioluminescent imaging data (n=5) for treatment with vaccinia strain only (WR TK-only), vaccinia strain and MMP8 expression (WR TK-mmp8), or vaccinia strain and PH20 expression (WR TK-ph20). Expression of PH20 resulted in increased viral spread.

[0009] **FIGURE 9** shows the effect of treatment with vaccinia virus containing the exemplary variant HMGB1 (HMGB1mut) alone or in combination with PH20 expression on tumor volume. Athymic nu/nu mice bearing subcutaneous MDA-MB-231 tumors (50-100 mm^3) were treated with the viruses indicated via a single intravenous treatment using 1E7 plaque-forming units (PFU). Tumor volume shown was measured at 14 days post treatment.

[0010] **FIGURE 10** shows the effect of virus expressing HMGB1mut and PH20 on tumor volume. Mice (BALB/c) bearing subcutaneous Renca tumors (50-100 mm^3) were treated (n=10/group) at day 0 with a single intravenous injection of vehicle as a control, 1E8 PFU of virus expressing HMGB1mut and PH20 (WO-H1), or 1E8 PFU of a mimetic of the best in class

clinical virus Pexa-Vec, also known as JX-594 (WR.TK-GMCSF+). Tumor volume was measured as a function of days post treatment.

[0011] **FIGURE 11** shows the effect of virus expressing HMGB1mut and PH20 on tumor volume in a model of peritoneal cancer in obese mice. Mice (C57/BL6) were fed a high fat diet until body weight increased >10% over control mice fed a normal diet. Mice were then implanted with MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity. Tumor growth was followed by bioluminescence imaging. Once tumors were established, mice were treated with a single, low dose intraperitoneal injection of PBS (control), 5E6 PFU of virus expressing HMGB1mut and PH20 (WO-H1), or 5E6 PFU of a mimetic of the best in class clinical virus Pexa-Vec, also known as JX-594 (WR.TK-GMCSF+).

[0012] **FIGURE 12** shows the effect of virus expressing HMGB1mut and PH20 on tumor volume in mice fed a ketogenic diet. Mice (C57/BL6) were implanted with MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity. Tumor growth was followed by bioluminescence imaging. Once tumors were established, mice were treated with a period of 24h starvation followed by low dose (5E6 PFU intraperitoneal) virus treatment with WO-H1 (Starve-virus), or virus alone.

DETAILED DESCRIPTION

[0022] 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

[0023] 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.”

[0024] 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".

[0025] The terms "individual," "patient," or "subject" are 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.

[0026] The terms "heterologous nucleic acid sequence," or "exogenous nucleic acid sequence," 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.

[0027] 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.

[0028] 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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).

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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).

[0038] 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.

[0039] 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.

[0040] The term "oncolytic," as used herein, can refer to killing of cancer or tumor cells by an agent, 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," refers to killing of cancer or tumor cells without lysis of said cells.

Modified Viruses

[0041] In some embodiments are provided modified viruses, *e.g.*, oncolytic vaccinia viruses that can contain an exogenous nucleic acid sequence that can code for a variant HMGB1 protein. Any other oncolytic virus can also be modified by inserting any of the exogenous nucleic acid sequences disclosed herein, to express a variant HMGB1, alone or in combination with additional exogenous nucleic acid sequences, further deletions of viral genes, *e.g.*, virulence genes, or any combinations thereof. Examples of viruses included, but are not limited to, Herpes Simplex Virus (HSV), Adenovirus, Polio virus, VSV, Coxsackievirus, reovirus, lentivirus, adeno-associated virus (AAV), Measles virus, Maraba virus, Newcastle disease (NDV), Seneca valley virus, Reovirus, Mengovirus, and Myxomavir.

[0042] Viruses described herein comprise one or more exogenous nucleic acid sequences, alternatively referred to as transgenes, which can generate mRNAs coding for a variant HMGB1 protein (also referred to herein an HMGB1mut). Structurally, the HMGB1 protein is

approximately 25kDa with a highly and can comprise a structure composed of multiple domains, such as a box A domain, a box B domain, a C-terminal acidic tail, and a domain formed by the region between box B and the C-terminal acidic tail. Within the box A, the HMGB1 protein can comprise a first nuclear localization signal (NLS1) and within the domain formed by the region between box B and the C-terminal acidic tail, the HMGB1 protein can comprise a second nuclear localization signal (NLS2). Fig. 1(a) shows an exemplary structural arrangement for the HMGB1 protein. In certain embodiments of this disclosure is described, a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1, wherein said variant HMGB1 comprises a mutation in at least one of NLS1 and NLS2. The exogenous nucleic acid can comprise a further mutation in at least one of the domains described above as box A, box B, or the domain formed by the region between box B and the C-terminal acidic tail, or any combinations thereof. In some examples, one or more mutations in box A can be associated with a cytoplasmic relocation of the variant HMGB1. In some cases, the exogenous nucleic acid may not comprise the C-terminal acidic tail domain.

[0043] The cytoplasmic relocation can be attributed to one or more mutations in the NLS1 within box A, or one or more mutations in the NLS2 located in the domain formed by the region between the box B and the C-terminal acidic tail, or one or more mutations in both the NLS1 within box A and the NLS2 located in the domain formed by the region between the box B and the C-terminal acidic tail. A variant HMGB1 that relocates to the cytoplasm can be advantageous in inducing a cytotoxic immune response in tumor cells that have been infected with an oncolytic vaccinia virus that can comprise an exogenous nucleic acid sequence that can code for the variant HMGB1, as well as cells that are in the vicinity of the infected tumor cell but may not be directly infected by the vaccinia virus. Thus, the oncolytic vaccinia virus of this disclosure, comprising an exogenous nucleic acid that codes for a variant HMGB1 can be more efficacious as an inducer of cytotoxic immune response in a tumor microenvironment, generates enhanced cytotoxic immune response in uninfected cells by a bystander effect, compared to an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid coding for a variant HMGB1.

[0044] In some examples, the mutations present in a variant HMGB1 as described herein, *e.g.*, one or more mutations in at least one of the following domains: box A box B, the domain formed by the region between box B and the acidic C-terminal tail; NLS1 within box A, NLS2 within the domain formed by the region between box B and the acidic C-terminal tail, or any combinations thereof, can be associated with an enhanced synergistic effect, when the virus, *e.g.*, an oncolytic vaccinia virus comprising the exogenous nucleic acid that

codes for the variant HMGB1 is administered in combination with a chemotherapy; said synergistic effect is not seen upon administration of an otherwise identical vaccinia virus that lacks the exogenous nucleic acid sequence coding for the variant HMGB1 that can comprise a mutation in at least one of the following domains: box A, box B, the domain formed by the region between box B and the acidic C-terminal tail; NLS1 within box A, NLS2 within the domain formed by the region between box B and the acidic C-terminal tail, or any combinations thereof. The synergistic effect of a combination with chemotherapy can be mediated by an immune effect and can be, in some cases, attributed to enhanced binding of the variant HMGB1 to at least one of TLR4 and RAGE.

[0045] In some examples, the mutations present in a variant HMGB1 as described herein, *e.g.*, one or more mutations in at least one of the following domains: box A box B, the domain formed by the region between box B and the acidic C-terminal tail; NLS1 within box A, NLS2 within the domain formed by the region between box B and the acidic C-terminal tail, or any combinations thereof, can be associated with increased tumor selective replication of a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise the exogenous nucleic acid that can code for the variant HMGB1; said increase in tumor selective replication is not seen upon administration of an otherwise identical vaccinia virus that lacks the exogenous nucleic acid sequence coding for the variant HMGB1 that can comprise a mutation in at least one of the following domains: box A, box B, the domain formed by the region between box B and the acidic C-terminal tail; NLS1 within box A, NLS2 within the domain formed by the region between box B and the acidic C-terminal tail, or any combinations thereof. The increased tumor specific viral replication, in some examples, can be mediated by the effect of the variant HMGB1 on autophagy, and in some cases, attributed to certain cysteine residues within the variant HMGB1 protein.

[0046] In some examples, the mutations present in a variant HMGB1 as described herein, *e.g.*, one or more mutations in at least one of the following domains: box A box B, the domain formed by the region between box B and the acidic C-terminal tail; NLS1 within box A, NLS2 within the domain formed by the region between box B and the acidic C-terminal tail, or any combinations thereof, can be associated with reduction in tumor growth by administering a, *e.g.*, an oncolytic vaccinia virus, that can comprise the exogenous nucleic acid that can code for the variant HMGB1, which lowers tumor interstitial fluid pressure (TIFP); said reduction in tumor growth by lowering of TIFP is not seen upon administration of an otherwise identical vaccinia virus that lacks the exogenous nucleic acid sequence coding for the variant HMGB1 that can comprise a mutation in at least one of the following domains: box A, box B, the domain formed by the region between box B and the acidic C-

terminal tail; NLS1 within box A, NLS2 within the domain formed by the region between box B and the acidic C-terminal tail, or any combinations thereof.

[0047] The variant HMGB1, in some embodiments, can comprise an amino acid sequence that is less than about or equal to 80%, less than about or equal to 81%, less than about or equal to 82%, less than about or equal to 83%, less than about or equal to 84%, less than about or equal to 85%, less than about or equal to 86%, less than about or equal to 87%, less than about or equal to 88%, less than about or equal to 89%, less than about or equal to 90%, less than about or equal to 91%, less than about or equal to 92%, less than about or equal to 93%, less than about or equal to 94%, less than about or equal to 95%, less than about or equal to 96%, less than about or equal to 97%, less than about or equal to 98%, or less than about or equal to 99%, homologous to SEQ ID NO: 16, which corresponds to human wild type HMGB1 (also referred to herein as HMGB1wt).

[0048] Within the amino acid sequence set forth as SEQ ID NO: 16, various domains of HMGB1wt can be delineated as follows: box A can comprises residues 1-85; box B can comprise residues 88-162, C-terminal acidic tail can comprise residues 186-215; the domain formed between box B and the C-terminal acidic tail can comprise residues 162-185; NLS1 can comprise residues 28-54; and NLS2 can comprise residues 179-185. Some examples of the virus can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1, which variant HMGB1 can be less than about or equal to 95% homologous to SEQ ID NO: 16, and can comprises at least one of residues 1-85, 89-108, and 150-183, wherein said at least one of residues 1-85, 89-108, and 150-183 can comprise at least one mutation compared to the amino acid sequence of SEQ ID NO: 16. For example, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise residues 1-85 of SEQ ID NO: 16, wherein one or more serine residue within the stretch can be mutated, for example, to alanine. Furthermore, in some examples, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise residues 89-108 of SEQ ID NO: 16, wherein one or more phenyl alanine residue within the stretch can be mutated, for example, to glutamic acid. In yet other examples, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise residues 150-183 of SEQ ID NO: 16, wherein a serine residue within the stretch can be mutated, for example, to alanine. In certain instances, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise mutations at one or more of positions 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16. In other instances, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an

exogenous nucleic acid sequence that can comprise mutations at all of positions 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

[0049] One embodiment provides a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1 that is less than about or equal to 95% homologous to SEQ ID NO: 16 and can comprises at least one of residues 23, 45, 23-45, 89-108, and 150-183 of SEQ ID NO: 16. In case of such viruses, the amino acid at positions 23 and 45 are both cysteines. Regarding the stretches covered by residues 89-108 and 150-183, the exogenous nucleic acid coding for variant HMGB1, as described herein, can comprise at least one mutation in those stretches compared to the amino acid SEQ ID NO: 16. For example, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise residues 89-108 of SEQ ID NO: 16, wherein one or more phenyl alanine residue within the stretch can be mutated, for example, to glutamic acid. In yet other examples, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise residues 150-183 of SEQ ID NO: 16, wherein a serine residue within the stretch can be mutated, for example, to alanine. In certain instances, the virus, *e.g.*, an oncolytic vaccinia virus, can comprise an exogenous nucleic acid sequence that can comprise mutations at one or more of positions 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16. In other instances, the virus, *e.g.*, an oncolytic vaccinia virus can comprise an exogenous nucleic acid sequence that can comprise mutations at all of positions 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16. In some instances, the exogenous nucleic acid comprises residues 23-54 of SEQ ID NO: 16, wherein amino acids at positions 23 and 45 can be cysteines and one or more of the serine residue in that stretch can be mutated to, *e.g.*, to alanine.

[0050] A further embodiment provides a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1 that can be at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof. The virus, as described above, can comprise at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15. In some cases, the virus can comprise at least one of residues 21-105, 109-128, and 170-203 of SEQ ID NO: 15. The stretch covered by residues 109-128 and 170-183 can play a role in binding of the HMGB1 variant to TLR4 and RAGE, respectively. Binding of the variant HMGB1 to at least one of TLR4 and RAGE can be correlated with an immune effect which can lead to , when the virus, *e.g.*, an oncolytic vaccinia virus, comprising the exogenous nucleic acid that codes for the variant

HMGB1 is administered in combination with chemotherapy. The amino acids in positions 43 and 65 of SEQ ID NO: 15 can be cysteines. In certain examples, the variant HMGB1, in its mature form, may not comprise residues 1-20 of SEQ ID NO: 15. A "fragment" of a nucleic acid or an amino acid described herein can refer to a portion comprising about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% of the entire length of the nucleic acid of amino acid sequence of which it is a fragment.

[0051] A further embodiment provides a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1 that can be at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 17, or a fragment thereof. The virus, as described above, can comprise at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15. In some cases, the virus can comprise at least one of residues 21-105, 109-128, and 170-203 of SEQ ID NO: 17. The stretch covered by residues 109-128 and 170-183 can play a role in binding of the HMGB1 variant to TLR4 and RAGE, respectively. Binding of the variant HMGB1 to at least one of TLR4 and RAGE can be correlated with an immune effect which can mediate an enhanced synergistic effect, when the virus, *e.g.*, an oncolytic vaccinia virus, comprising the exogenous nucleic acid that codes for the variant HMGB1 is administered in combination with chemotherapy. The amino acids in positions 43 and 65 of SEQ ID NO: 17 can be cysteines. The amino acids in positions 43 and 65 of SEQ ID NO: 15 can be cysteines. In certain examples, the variant HMGB1, in its mature form, may not comprise residues 1-20 of SEQ ID NO: 17.

[0052] A further embodiment provides a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1 that can be at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 4, or a fragment thereof. The virus, as described above, can comprise at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 4. In some cases, the virus can comprise at least one of residues 21-105, 109-128, and 170-203 of SEQ ID NO: 4. The stretch covered by residues 109-128 and 170-183 can play a role in binding of the HMGB1 variant to TLR4 and RAGE, respectively. Binding of the variant HMGB1 to at least one of TLR4 and RAGE can be correlated with an immune effect which can mediate an enhanced synergistic effect, when the virus, *e.g.*, an oncolytic vaccinia virus, comprising the exogenous nucleic acid that codes for the variant HMGB1 is administered in combination with chemotherapy. The amino acids in positions 43 and 65 of SEQ ID NO: 4 can be cysteines. The amino acids in

positions 43 and 65 of SEQ ID NO: 4 can be cysteines. In certain examples, the variant HMGB1, in its mature form, may not comprise residues 1-20 of SEQ ID NO: 4.

[0053] A further embodiment provides a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1 that can be at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to any one of SEQ ID NOs: 5, 6, 22-23, or a fragment thereof. In some cases, the coding sequence for a variant HMGB1 can be fused, upstream, to a coding sequence for an IgE leader sequence. The resulting protein can comprise an IgE leader sequence followed by a variant HMGB1 sequence, as exemplified in SEQ ID NOs: 24 and 25.

[0054] Within the amino acid sequences set forth as SEQ ID NOs: 4, 15, and 17, various domains of HMGB1 can be delineated as follows: box A can comprises residues 21-105; box B can comprise residues 108-182, C-terminal acidic tail can comprise residues 206-235; the domain formed between box B and the C-terminal acidic tail can comprise residues 183-205; NLS1 can comprise residues 47-74; and NLS2 can comprise residues 199-205.

[0055] A further embodiment provides a virus, *e.g.*, an oncolytic vaccinia virus, that can comprise an exogenous nucleic acid sequence that can code for a variant HMGB1 that can be at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to any one of SEQ ID NOs: 24-25, or a fragment thereof.

[0056] Within the amino acid sequences set forth as SEQ ID NOs: 22 and 23, various domains of HMGB1 can be delineated as follows: box A can comprises residues 1-85; box B can comprise residues 88-162, C-terminal acidic tail can comprise residues 186-215; the domain formed between box B and the C-terminal acidic tail can comprise residues 162-185; NLS1 can comprise residues 28-54; and NLS2 can comprise residues 179-185.

[0057] Within the amino acid sequences set forth as SEQ ID NOs: 24 and 25, various domains of HMGB1 can be delineated as follows: box A can comprises residues 19-103; box B can comprise residues 106-180, C-terminal acidic tail can comprise residues 204-233; the domain formed between box B and the C-terminal acidic tail can comprise residues 181-203; NLS1 can comprise residues 45-72; and NLS2 can comprise residues 197-203.

[0058] Presence of cysteine residues in positions 23 and 45 of an exogenous nucleic acid which is less than about or equal to 95% homologous to SEQ ID NO: 16, and positions 43 and 65 of an exogenous nucleic acid which is at least about 80% homologous any one of SEQ ID NOs: 4, 15, and 17, can, in some cases, be correlated to increase in tumor specific replication of viruses, *e.g.*, oncolytic vaccinia viruses, which may contain one of said

exogenous nucleic acid sequences that codes for a variant HMGB1. The increased tumor specific replication of oncolytic vaccinia viruses expressing the variant HMGB1 proteins can be mediated by the effect of the HMGB1 protein on autophagy, and the cysteine residues identified above may play a role in the same.

[0059] The exogenous nucleic acid coding for the variant HMGB1 can comprise a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to any one of SEQ ID NO: 13, SEQ ID NO: 13, 2, 3, 7, 9, 14, and 19. In some examples, the exogenous nucleic acid comprises a nucleic acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to any one of SEQ ID NO: 13 and wherein said nucleic acid codes for a variant HMGB1 that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to any one of SEQ ID NO: 15.

[0060] One embodiment describes a virus, *e.g.*, an oncolytic virus that can further express a hyaluronidase in addition to the variant HMGB1. The hyaluronidase can be coded by a further exogenous nucleic acid sequence within the oncolytic vaccinia virus; said further exogenous nucleic acid sequence can have a nucleotide sequence set forth in SEQ ID NO: 11, and the hyaluronidase expressed from the oncolytic vaccinia virus can have an amino acid sequence as set forth in SEQ ID NO: 12. Any hyaluronidase can be expressed from the virus, including, but not limited to, HYAL1, HYAL2, and HYAL3, HYAL4, PH-20, SPAM1 (sperm adhesion molecule 1), and HYALP1.

[0061] One embodiment describes a virus, *e.g.*, an oncolytic virus that can further express a protein that stabilizes the virus in circulation, in addition to the variant HMGB1. Examples of such protein can include IgE, an Fc domain protein. In some cases, the IgE can be coded by a further exogenous nucleic acid sequence within the oncolytic vaccinia virus; said IgE expressed from the oncolytic vaccinia virus can have an amino acid sequence as set forth in SEQ ID NO: 18.

[0062] The exogenous nucleic acid sequence coding for a hyaluronidase, and the sequence coding for IgE can be operably linked to the exogenous nucleic acid sequence coding for a variant HMGB1 as described herein. In some examples, the IgE is fused upstream to both variant HMGB1 coding gene and the hyaluronidase coding gene. Other arrangements of the IgE, hyaluronidase, and variant HMGB1 coding gene are also contemplated.

[0063] Additional modifications to the virus, *e.g.*, to an oncolytic vaccinia virus can include, but are not limited to, deletions of virulence genes, such as *B8R* (codes for IFN-g binding protein),

B18R (codes for type I IFN binding protein), *B13R* (codes for serpin *SPI-1* which has anti-apoptotic activity, *B22R* (codes for serpin *SPI-2* which has anti-apoptotic activity), *B15R* (codes for IL-1b binding protein), *VGF* (codes for Vaccinia Growth Factor), *E3L* (codes for dsRNA binding), *K3L* (codes for PKR binding).

[0064] The modified virus can further express a chemotherapy sensitizer, such as hyaluronic acid; an apoptosis enhancer, such as TRAIL or caspase. In some cases, the modified virus can further express an efflux pump blockers, such as a multi drug transporter inhibitor.

[0065] In certain embodiments, exogenous nucleic acid sequences for insertion into the viral genome, *e.g.*, in the thymidine kinase locus or in place of other deletions within the genome of an oncolytic vaccinia virus, can be codon optimized. Further, the variant HMGB1 expressed by such exogenous nucleic acid sequences, as well as the hyaluronidase and stabilizing protein sequences can have variants such that the resulting protein maintains their respective functions as described herein. In certain embodiments, such changes are referred to as conservative substitutions. As used herein, the terms "conservative substitutions" and "conservative modifications" refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the presently disclosed variant HMGB1 proteins comprising the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into the HMGB1 proteins of this disclosure by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Amino acids can be classified into groups according to their physicochemical properties such as charge and polarity.

[0066] Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid within the same group. For example, amino acids can be classified by charge: positively-charged amino acids include lysine, arginine, histidine, negatively-charged amino acids include aspartic acid, glutamic acid, neutral charge amino acids include alanine, asparagine, cysteine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In addition, amino acids can be classified by polarity: polar amino acids include arginine (basic polar), asparagine, aspartic acid (acidic polar), glutamic acid (acidic polar), glutamine, histidine (basic polar), lysine (basic polar), serine, threonine, and tyrosine; non-polar amino acids include alanine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and valine. Thus, one or more amino acid residues within the variant HMGB1 protein can be replaced with other amino acid residues from the same group and the altered variant HMGB1 protein can be tested for retained function using the functional assays

described herein. In certain embodiments, no more than one, no more than two, no more than three, no more than four, no more than five residues within a specified sequence are altered.

[0067] Exemplary conservative amino acid substitutions are shown in Table I.

Table I

Original Residue	Exemplary Conservative Amino Acid Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gin; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Giu; Asn
Cys (C)	Ser; Ala
Gin (Q)	Asn; Glu
Glu (E)	Asp; Gin
Gly (G)	Ala
His (H)	Asn; Gin; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe
Leu (L)	Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gin; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala

Cancer Targets

[0068] 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 virus, such as an oncolytic vaccinia virus as described herein, is contemplated. Cancers that can be treated by a modified virus, *e.g.*, an oncolytic vaccinia virus that can comprise an exogenous nucleic acid coding for a variant HMGB1 protein, 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, 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, and sarcoma.

[0069] 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 various examples, the variant HMGB1for use in a modified virus of this disclosure, such as an oncolytic vaccinia virus, that can be used to treat cancer targets disclosed herein, can be a variant HMGB1 comprising the sequence set forth as SEQ ID NO: 4, 15, 17, 22, 23, 24, or 25.

[0070] 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 variant HMGB1 proteins that can be encoded by a modified virus, such as an oncolytic vaccinia virus, as discussed herein. In various examples, the variant HMGB1for use in a modified virus of this disclosure, such as an oncolytic vaccinia virus, that can be used for inhibiting or preventing local invasiveness or metastasis, or both, of any type of primary cancer, can be a variant HMGB1 comprising the sequence set forth as SEQ ID NO: 4, 15, 17, 22, 23, 24, or 25.

[0071] In some cases, the modified virus, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1 can be administered for treatment of a peritoneal cancer. Peritoneal cancer can refer to cancer of the peritoneum. The peritoneum can be defined as tissue that lines the abdominal wall and covers abdominal organs. Primary peritoneal cancer can refer to cancer that originated in the peritoneum and did not spread to the peritoneum from another part of the body.

[0072] Furthermore, the modified virus, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1 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 virus within the tumor. 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 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 virus within the tumor. The modified virus, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1, can form an increased titer of extracellular enveloped virus (EEV) in tumors with high bioavailability of free fatty acids.

[0073] Exemplary cancer patient populations that can benefit from the modified virus of this disclosure, such as an oncolytic vaccinia virus that expresses a variant HMGB1, optionally with a hyaluronidase, *e.g.*, PH-20, include, but are not limited to, patients who are obese, patients who are on ketogenic diet or short term fasting, patients who have peritoneal cancer, patients who have primary peritoneal cancer, patients who have peritoneal cancer affecting the uterus, bladder, and rectum. Further, patients that have previously failed immunotherapy can be identified as a target population for treatment using the modified viruses described herein, according to the methods disclosed herein. Such patients may not benefit from a further round of immunotherapy and can move on to a treatment with a modified virus, such

as an oncolytic vaccinia virus of this disclosure, alone or in combination with chemotherapy. Thus, therapy using the modified viruses of this disclosure can be a key alternative in case of patients who have failed immunotherapy.

[0074] The term “obese” or “obesity,” can refer to a condition wherein an individual has a BMI equal to or greater than 30 kg/m2. According to a World Health Organization (WHO) definition, obesity can be categorized as follows: the term “class I obesity” can be a condition wherein the BMI is equal to or greater than 30 kg/m2 but lower than 35 kg/m2; the term “class II obesity” can be a condition wherein the BMI is equal to or greater than 35 kg/m2 but lower than 40 kg/m2; the term “class III obesity” can be a condition wherein the BMI is equal to or greater than 40 kg/m2. A BMI of 18.5 to 24.9 kg/m2 can be classified as normal, and a BMI of 25.0 to 29.9 kg/m2 can be classified as overweight. The term “body mass index” or “BMI” can be defined as weight in kilograms divided by height in meters squared, such that BMI has units of kg/m2. BMI can be a measure for defining obesity.

Methods of treatment and assaying the efficacy and pharmacokinetics

[0075] This disclosure provides methods for treating a subject by administration of one or more modified 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.

[0076] 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 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.

[0077] 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 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.

[0078] 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 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.

[0079] 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 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 virus of this disclosure, *e.g.*, an oncolytic vaccinia virus containing the exogenous nucleic acid that codes for a variant HMGB1 as described herein, compared to that in an otherwise identical virus that does not contain the exogenous nucleic acid sequence coding for the variant HMGB1. Other exemplary techniques for detecting and monitoring viral load after administration of the modified viruses include real-time quantitative PCR.

[0080] Further provided is a method of monitoring the pharmacokinetics following administration of a therapeutically effective amount of modified 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 virus of the present disclosure, such as an oncolytic vaccinia virus as described herein.

[0081] 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 virus of this disclosure, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1, compared to that in an otherwise identical oncolytic vaccinia virus that does not contain the exogenous nucleic acid sequence that codes for the variant HMGB1.

[0082] The hyaluronidase PH-2O can function in extracellular matrix degradation, which can improve viral spread. Viral spread can also be measured through use of a reporter gene, such as a luciferase gene. 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 evaluate viral spread. Some examples show an increase in luciferase reporter bioluminescence in a modified virus of this disclosure, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1 in combination with the PH-20, compared to that in an otherwise identical oncolytic vaccinia virus that does not contain the exogenous nucleic acid sequence that codes for the variant HMGB1 in combination with the PH-20. Thus, in certain instances, the modified virus of this disclosure, such as an as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1 alone, or in combination with PH-20, demonstrate increased tumor specific viral replication and improved viral spread compared to viruses which do not have the exogenous genes as mentioned above.

Delivery of modified viruses

[0083] In some embodiments, amount of a modified 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 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 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 virus of this disclosure, such as an oncolytic vaccinia virus, can be administered at a dose that can comprise about 2×10^3 PFU/dose, 3×10^3 PFU/dose , 4×10^3 PFU/dose , 5×10^3 PFU/dose , 6×10^3 PFU/dose , 7×10^3 PFU/dose , 8×10^3 PFU/dose , 9×10^3 PFU/dose , about 10^4 PFU/dose, about 2×10^4 PFU/dose, about 3×10^4 PFU/dose , about 4×10^4 PFU/dose , about 5×10^4 PFU/dose , about 6×10^4 PFU/dose , about 7×10^4 PFU/dose , about 8×10^4 PFU/dose , about 9×10^4 PFU/dose , about 10^5 PFU/dose, 2×10^5 PFU/dose , 3×10^5 PFU/dose , 4×10^5 PFU/dose , 5×10^5 PFU/dose , 6×10^5 PFU/dose , 7×10^5 PFU/dose , 8×10^5 PFU/dose , 9×10^5 PFU/dose , about 10^6 PFU/dose, about 2×10^6 PFU/dose , about 3×10^6 PFU/dose , about 4×10^6 PFU/dose , about 5×10^6 PFU/dose , about 6×10^6 PFU/dose , about 7×10^6 PFU/dose , about 8×10^6 PFU/dose , about 9×10^6 PFU/dose , about 10^7 PFU/dose, about 2×10^7 PFU/dose, about 3×10^7 PFU/dose, about 4×10^7 PFU/dose, about 5×10^7 PFU/dose, about 6×10^7 PFU/dose, about 7×10^7 PFU/dose, about 8×10^7 PFU/dose, about 9×10^7 PFU/dose, about 10^8 PFU/dose, about 2×10^8 PFU/dose, about 3×10^8 PFU/dose , about 4×10^8 PFU/dose , about 5×10^8 PFU/dose , about 6×10^8 PFU/dose , about 7×10^8 PFU/dose , about 8×10^8 PFU/dose , about 9×10^8 PFU/dose , about 10^9 PFU/dose, about 2×10^9 PFU/dose, about 3×10^9 PFU/dose, about 4×10^9 PFU/dose, about 5×10^9 PFU/dose, about 6×10^9 PFU/dose, about 7×10^9 PFU/dose, about 8×10^9 PFU/dose, about 9×10^9 PFU/dose, about 10^{10} PFU/dose, about 2×10^{10} PFU/dose, about 3×10^{10} PFU/dose, about 4×10^{10} PFU/dose, about 5×10^{10} PFU/dose , about 6×10^{10} PFU/dose, about 7×10^{10} PFU/dose , about 8×10^{10} PFU/dose , about 9×10^{10} PFU/dose, about 10^{10} PFU/dose, about 2×10^{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 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 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.

[0084] In some embodiments, a modified 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.

[0085] In some embodiments, a modified 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 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 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 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.

[0086] In some embodiments, a modified 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.

[0087] 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 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 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 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.

[0088] In some instances, where the modified 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 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, 2×10^{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 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, $3x10^8$ $TCID_{50}$/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 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 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 vaccina 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.

[0089] 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 viruses, such as the oncolytic vaccina 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.

[0090] An exemplary method for the delivery of a modified 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 virus can be administered to the patient from a source implanted in the patient. In certain embodiments, administration of the modified 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

[0091] 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 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.

[0092] 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 glucomate, 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.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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, pecitin, 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.

[0097] 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.

[0098] 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.

[0099] 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.

[00100] 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.

[00101] Also contemplated are combination products that include one or more modified 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 nysatin, 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.

[00102] 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.

[00103] 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.

[00104] In certain embodiments, sterile injectable solutions can be prepared by incorporating a modified 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.

[00105] 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 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

[00106] The modified viruses of this disclosure can be produced by methods known to one of skill in the art. In certain embodiments, the modified 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 viruses are propagated in host cells using cell stacks, roller bottles, or perfusion bioreactors. In some examples, downstream methods for purification of the modified viruses can comprise filtration (*e.g.*, depth filtration, tangential flow filtration, or a combination thereof), ultracentrifugation, or chromatographic capture. The modified virus can be stored, *e.g.*, by freezing or drying, such as by lyophilization. In certain embodiments, prior to administration, the stored modified virus can be reconstituted (if dried for storage) and diluted in a pharmaceutically acceptable carrier for administration.

[00107] Some embodiments provide that the modified virus as described herein, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid coding for variant HMGB1 exhibit a higher titer in HeLa cells and 293 cells compared to an otherwise identical virus that does not contain the exogenous nucleic acid coding for the variant HMGB1. In certain

instances, a higher titer in HeLa cells and 293 cells is seen in modified vaccinia viruses expressing a variant HMGB1 and a hyaluronidase, such as PH-20.

Combination therapies

[00108] In certain embodiments, the methods of this disclosure comprise administering a modified 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 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 virus of this disclosure, such as an oncolytic vaccinia virus comprising an exogenous nucleic acid sequence that codes for a variant HMGB1 as described herein, alone or in combination with PH-20. Combination of the oncolytic vaccinia virus with chemotherapy achieves a synergistic effect which is not seen in modified viruses that do not contain the variant HMGB1 coding nucleic acid sequence. 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%.

[00109] In certain embodiments, treatment using a modified 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 1FNy, and chemokines, such as MIP-1, MCP-1 and IL-8. In certain embodiments, the immunomodulatory agent includes immune checkpoint inhibitors such as, but not limited to, anti-CTLA4, anti-PD-1, anti-PDL1 and TLR agonists *(e.g.,* Poly 1:C).

[00110] 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."

[00111] 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.

[00112] 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®).

[00113] 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.

[00114] 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®).

[00115] Exemplary proteosome 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).

[00116] "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 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 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.

[00117] 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 virus, *e.g.,* vaccinia virus, expressing one or more nucleic acids that encode variant HMGB1 protein comprising an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 15, 17, 19, 5, 6, 8, 10, and 22-25 and conservative substitutions thereof. For example, and not by way of limitation, a modified virus of this disclosure can comprise a nucleic acid that can comprise a nucleotide sequence that is at least about 85%, about 90%, about 95%,about 96%, about 97%, about 98%, about 99% or 100% homologous to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 8-10. 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

[00118] In embodiments, this disclosure provides for a kit for administering a modified virus as described herein. In certain embodiments, a kit of this disclosure can include a modified virus or a pharmaceutical composition comprising a modified 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 combination thereof, that can be administered in combination with a modified virus.

[00119] 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 vaccinia virus expressing a variant HMGB1, with or without at least one of PH20 and IgE, or any combinations thereof. In certain embodiments, the variant HMGB1 can be conjugated to a cell penetrating peptide.

[00120] In certain embodiments, a kit of this disclosure can include an effective amount of a modified vaccinia virus comprising one or more nucleic acids that encode a variant HMGB1 that can comprise an amino acid sequence that can be at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% homologous to an amino acid sequence selected from the group consisting of SEQ ID

NOs: 4, 15, 17, and 22-25, and conservative substitutions thereof. In certain embodiments, a kit of this disclosure can include an effective amount of a modified vaccinia virus comprising one or more nucleic acids that encode a variant HMGB1 that can comprise an amino acid sequence that can be less than about or equal to 85%, less than about or equal to 90%,, less than about or equal to 95%, less than about or equal to 96%, less than about or equal to 97%, or less than about or equal to 98%, or less than about or equal to 99% homologous to SEQ ID NO: 16, and conservative substitutions thereof.

[00121] In certain embodiments, a kit of this disclosure can include instructions for use, a device for administering the modified 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 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.

[00122] In certain embodiments, a kit of this disclosure can include a device for administering the modified 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 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

[00123] The examples below further illustrate the described embodiments without limiting the scope of this disclosure.

[00124] **EXAMPLE 1: DESIGN AND EXPRESSION OF AN EXEMPLARY VARIANT HMGB1**

[00125] One example of a variant HMGB1 (also referred to herein as "HMGB1mut") that provided unexpected therapeutic advantage is shown in Fig 1a. This diagram shows that the two nuclear localization signals (NLSs) in HMGB1 were mutated, and an IgE sequence was fused to the N-terminus of the transgene to stabilize the protein in the extracellular environment. This

increased the variant HMGB1's immune-activating functions to allow greater bystander effect, with uptake in surrounding, uninfected cells leading to enhanced autophagy.

[00126] This transgene was inserted into the viral thymidine kinase locus under control of the viral p7.5 promoter (Fig lb). Loss of thymidine kinase function is a well-characterized approach to creating a tumor-selective VACV vector. In addition, luciferase was inserted into the same locus under control of the pSE/L promoter and used as a reporter gene to track viral gene expression in vitro and in vivo. Expression of HMGBlmut was confirmed by Western Blot (Fig 1c) and ELISA (Fig 1d) in the media from two mouse tumor cell lines (Renca and 4T1) after infection with different viruses.

[00127] **EXAMPLE 2: VIRAL EXPRESSION OF THE EXEMPLARY VARIANT HMGB1 ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00128] The aim of this study was to explore the effects of an exemplary modified oncolytic vaccinia virus according to this disclosure, where the thymidine kinase gene was deleted and an exogenous nucleic acid encoding a variant HMGB1 was added (referred to herein as TK-HMGB1mut), in cancer cell lines and murine models, in comparison with an oncolytic vaccinia virus that did not contain the nucleic acid expressing HMGB1mut (referred to herein as TK-).

[00129] In initial *in vivo* experiments, a single intravenous dose of virus (TK-or TK-HMGB1mut) was used to treat immunocompetent mice with pre-established subcutaneous tumors (Fig 2). It was seen that in the normally resistant Renca tumor model, HMGB1mut expression resulted in significantly enhanced therapeutic activity. In the more sensitive MC38 model, HMGB1mut expression provided an additional and significant therapeutic benefit; however, this was less dramatic.

[00130] The effects of HMGB1mut expression was examined across a range of mouse tumor and normal cell lines to better define the mechanisms driving the enhanced therapeutic activity. It was initially noted that phosphorylated IKKb levels were significantly increased with HMGB1mut expression (Fig 3a). This would be expected to result in NF-kB activation, which in turn could sensitize cells to apoptosis and/or enhance release of a variety of cytokines. However, the expression levels of a variety of cytokines and chemokines involved in both innate and adaptive immunity were either unchanged or reduced after infection with virus expressing HMGB1mut (relative to infection with TK- virus) (Fig 3b-e). Furthermore, after naïve BALB/c mice were inoculated with either TK- or TK-HMGB1mut, there was no significant change in the level of anti-viral CTL response induced; in fact, there was a trend towards a reduction in the CTL response with HMGB1mut expression, although this was not significant (Fig 3f). Finally, when SCID mice bearing Renca tumors were treated with TK- or TK-HMGB1mut, the

HMGB1mut expression maintained a therapeutic advantage (Fig 3g). Therefore, there is a non-immune component to the therapeutic advantage provided by the transgene.

[00131] HMGB1 typically is associated with cell survival. Thus, reduced viral replication or no increase in viral replication was expected when using the HMGB1mut. However, one unexpected finding when using the HMGB1mut was an increase in viral replication that was seen across multiple tumor cell lines in culture (Fig 4a). This unexpected, increased replication was observed despite insignificant differences in cell killing (Fig 4b and 4d). HMGB1mut expression resulted in a small increase in cell killing, which combined with the increased activation of IKK (Fig 3a) cellular apoptosis.

[00132] The increased viral replication was also seen in tumors *in vivo* with significantly increased viral luciferase gene expression detected in both Renca (Fig 4c) and MC38 (Fig 4e) tumors. The increase was more significant in the Renca tumor model that also showed the greater increase in therapeutic benefit (Fig 2). It is notable that the increased replication (viral gene expression) seen *in vivo* was confined to the tumor (Fig 4f), even in SCID mice where the enhanced replication in the tumor (Fig 4g) is sustained continuously. This is notable as viral modifications that create tumor-targeting OV strains typically involve attenuating viral replication in normal tissues, rather than selectively enhancing replication in the tumor.

[00133] The effects of HMGBlmut expression on autophagy markers were examined. It was seen that expression of the transgene resulted in an increase in LC3 (Fig 5a) and that inhibitors of maturation of autophagic vacuoles (the vacuolar H+ATPase inhibitor, Bafilomycin A1) and PI3K (LY294002; PI3K/AKT/mTOR pathway is known to modulate autophagy) both resulted in loss of the increased viral gene expression originally seen with HMGB1mut expression (Fig 5b). This implies that HMGB1 effects on autophagy may drive the enhanced viral replication.

[00134] HCT 116 cells were transfected with siRNA targeting Atg5, a key mediator of the autophagy pathway. It was seen that loss of autophagic potential negatively affected viral replication (Fig 6a). This finding contradicts a previous study that indicated autophagy did not affect vaccinia replication.

[00135] The potential role of fatty acid synthase was examined for vaccinia replication. It was seen that the fatty acid synthase inhibitor cerulenin also significantly reduced vaccinia replication in tumor cells *in vitro* (Fig 6b).

[00136] The replication of TK-HMGBlmut benefited further from infection of tumors in models of obesity. The replication of both TK- and TK-HMGBlmut increased in the context of mice fed a high-fat diet (Fig 6c and 6d).

[00137] HMGB1mut synergizes with chemotherapies, including taxols and platinum-based drugs such as cisplatin. Media taken from cells infected with TK-HMGB1mut, filtered to remove any viral particles, and then placed on a fresh tumor cell layer sensitized these cells to subsequent exposure to chemotherapies, including cisplatin and taxol (Fig 7a). This effect was unexpectedly pronounced for viruses expressing HMGB1mut.

[00138] The effects were even more unexpectedly pronounced *in vivo* (Fig 7b). BALB/c mice bearing Renca tumors were treated with a single intravenous injection of viruses (TK- or TK-HMGB1mut) alone or in combination with paclitaxel (Taxol®). It was seen that TK-HMGBlmut plus Taxol® produced a significantly greater therapeutic effect than any other treatment.

[00139] The effects of vaccinia strain expression alone or in combination with matrix metalloproteinase-8 (MMP8) expression or expression of the hyaluronidase PH-2O on viral spread were evaluated in mice. Bioluminescent imaging of tumors in mice treated with a vaccinia strain only (WR TK-only), vaccinia strain and MMP8 expression (WR TK-mmp8), or vaccinia strain and PH20 expression (WR TK-ph20) revealed significantly increased viral spread in tumors with vaccinia strain and PH20 expression relative to the other conditions (Fig 8a and 8b).

[00140] I*n vivo* experiments further demonstrated the enhanced therapeutic effect of a combination of HMGB1mut and PH20 expression. Immunocompromised (athymic nu/nu) mice bearing pre-established subcutaneous MDA-MB-231 tumors (50-100 mm^3) were treated with a single intravenous dose of PBS (control) or virus (WR.TK-, WR.TK-PH20, or WR.TK-PH20.HMGB1mut), and tumor volume was measured. Treatment with WR.TK-PH20.HMGB1mut most significantly reduced tumor volume as measured 14 days post-treatment (Fig 9).

[00141] The therapeutic effect of the combination of HMGB1mut and PH20 expression was compared to a mimetic of the best in class clinical virus Pexa-Vec, also known as JX-594 (WR.TK-GMCSF+; Fig 10). BALB/c mice bearing subcutaneous Renca tumors (50-100 mm^3) were treated (n=10/group) at day 0 with a single intravenous injection of vehicle as a control, 1E8 PFU of virus expressing HMGB1mut and PH20 (WO-H1), or 1E8 PFU of virus expressing WR.TK-GMCSF+. Tumor volume was measured as a function of days post treatment. Treatment with HMGB1mut and PH20 resulted in reduced tumor volume relative to treatment with the Pexa-Vec mimetic (Fig 10).

[00142] The therapeutic effect of the combination of HMGB1mut and PH20 expression was also compared to the Pexa-Vec mimetic in a model of peritoneal cancer in obese mice. C57/BL6 mice were fed a high-fat diet until body weight increased >10% over control mice fed a normal diet. Mice were then implanted with MC-38 colorectal cancer cells expressing luciferase into the

peritoneal cavity. Tumor growth was followed by bioluminescence imaging. Once tumors were established (after 5 days), mice were treated with a single, low dose intraperitoneal injection of PBS (control), 5E6 PFU of virus expressing HMGB1mut and PH20, or 5E6 PFU of virus expressing the Pexa-Vec mimetic. Of note, the Pexa-Vec mimetic (WR.TK-.GMCSF+) actually resulted in slightly increased tumor growth, whereas the HMGB1mut and PH20 expressing virus (WO-H1) displayed significantly decreased tumor burden relative to PBS controls (Fig 11).

[00143] The therapeutic activity of the combination of HMGB1mut and PH20 expression was evaluated in the context of prior short term starvation. C57/BL6 mice were implanted with MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity. Tumor growth was followed by bioluminescence imaging. Once tumors were established (after 5 days), mice were treated with virus alone or with a period of 24h starvation followed by low dose (5E6 PFU intraperitoneal) HMGB1mut and PH20 expressing (WO-H1) virus (Fig 12). It was determined that, although WO-H1 displayed therapeutic activity in this model, this effect was enhanced through prior short term starvation, which was a pre-treatment designed to mimic a ketogenic diet and expected to increase autophagy in the tumor.

[00144] **EXAMPLE 3: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED ADENO ASSOCIATED VIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00145] The aim of this study is to explore the effects of an exemplary modified oncolytic adeno associated virus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with an adeno associated virus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified adeno associated virus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control adeno associated virus. Significantly reduced tumor burdens are observed in case of the test modified adeno associated viruses compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00146] **EXAMPLE 4: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED HERPES SIMPLEX VIRUS (HSV) ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00147] The aim of this study is to explore the effects of an exemplary modified oncolytic HSV, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is

inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a HSV that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified HSV of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control HSV. Significantly reduced tumor burdens are observed in case of the test modified HSV compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00148] **EXAMPLE 5: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED ADENOVIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00149] The aim of this study is to explore the effects of an exemplary modified oncolytic adenovirus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with an adenovirus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified adenovirus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control adenovirus. Significantly reduced tumor burdens are observed in case of the test modified adenovirus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00150] **EXAMPLE 6: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED VESICULAR STOMATITIS VIRUS (VSV) ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00151] The aim of this study is to explore the effects of an exemplary modified oncolytic VSV, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a VSV that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified VSV of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control VSV. Significantly reduced tumor burdens are observed in case of the test modified VSV compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are

subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00152] **EXAMPLE 6: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED POLIOVIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00153] The aim of this study is to explore the effects of an exemplary modified oncolytic poliovirus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a poliovirus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified poliovirus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control poliovirus. Significantly reduced tumor burdens are observed in case of the test modified poliovirus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00154] **EXAMPLE 7: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED NEWCASTLE DISEASE VIRUS (NDV) ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00155] The aim of this study is to explore the effects of an exemplary modified oncolytic NDV, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a NDV that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified NDV of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control NDV. Significantly reduced tumor burdens are observed in case of the test modified NDV compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00156] **EXAMPLE 8: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED SENECA VALLEY VIRUS (SVV) ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00157] The aim of this study is to explore the effects of an exemplary modified oncolytic SVV, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is

inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a SVV that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified SVV of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control SVV. Significantly reduced tumor burdens are observed in case of the test modified SVV compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00158] **EXAMPLE 9: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED COXSACKIEVIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00159] The aim of this study is to explore the effects of an exemplary modified oncolytic coxsackievirus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a coxsackievirus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified coxsackievirus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control coxsackievirus. Significantly reduced tumor burdens are observed in case of the test modified coxsackievirus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00160] **EXAMPLE 10: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED REOVIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00161] The aim of this study is to explore the effects of an exemplary modified oncolytic reovirus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a reovirus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified reovirus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control reovirus. Significantly reduced tumor burdens are observed in case of the test modified reovirus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control

mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00162] **EXAMPLE 11: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED LENTIVIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00163] The aim of this study is to explore the effects of an exemplary modified oncolytic lentivirus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a lentivirus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified lentivirus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control lentivirus. Significantly reduced tumor burdens are observed in case of the test modified lentivirus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00164] **EXAMPLE 12: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED MEASLES VIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00165] The aim of this study is to explore the effects of an exemplary modified oncolytic measles virus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a measles virus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified measles virus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control measles virus. Significantly reduced tumor burdens are observed in case of the test modified measles virus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00166] **EXAMPLE 13: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED MARABA VIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00167] The aim of this study is to explore the effects of an exemplary modified oncolytic maraba virus, according to this disclosure, where an exogenous nucleic acid encoding a variant

HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a maraba virus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified maraba virus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control maraba virus. Significantly reduced tumor burdens are observed in case of the test modified maraba virus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00168] **EXAMPLE 14: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED MYXOMAVIR VIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00169] The aim of this study is to explore the effects of an exemplary modified oncolytic myxomavir virus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a myxomavir virus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified myxomavir virus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control myxomavir virus. Significantly reduced tumor burdens are observed in case of the test modified myxomavir virus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00170] **EXAMPLE 15: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1 FROM A MODIFIED MENGOVIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00171] The aim of this study is to explore the effects of an exemplary modified oncolytic mengovirus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, alone or in combination with a further exogenous gene that codes for PH-20, in cancer cell lines and murine models, in comparison with a mengovirus that does not contain the nucleic acid expressing the variant HMGB1. It is observed that administration of the modified mengovirus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control mengovirus. Significantly reduced tumor burdens are observed in case of the test modified mengovirus compared to the

control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

[00172] **EXAMPLE 16: EXPRESSION OF AN EXEMPLARY VARIANT HMGB1, IN COMBINATION WITH A FURTHER VIRAL GENE MODIFICATION, FROM A MODIFIED VACCINIA VIRUS ENHANCES THERAPEUTIC EFFECTS IN CANCER CELL LINES AND MURINE TUMOR MODELS**

[00173] The aim of this study is to explore the effects of an exemplary modified oncolytic vaccinia virus, according to this disclosure, where an exogenous nucleic acid encoding a variant HMGB1 is inserted into the viral genome, in combination with a further exogenous gene that codes for PH-20, and a *B8R* gene deletion, in cancer cell lines and murine models, in comparison with an oncolytic vaccinia virus that does not contain the nucleic acids expressing the variant HMGB1 and PH-20, and *B8R* gene deletion. It is observed that administration of the modified oncolytic vaccinia virus of this disclosure, in combination with chemotherapy, results in a synergistic effect, compared to the control oncolytic vaccinia virus. Significantly reduced tumor burdens are observed in case of the test modified oncolytic vaccinia virus compared to the control virus, upon administration to mice that are fed a high-fat diet until body weight increases >10% over control mice fed a normal diet and are subsequently implanted MC-38 colorectal cancer cells expressing luciferase into the peritoneal cavity.

SEQUENCE LISTING TABLE:

SEQ ID NO: 1 (Nucleotide sequence coding for an exemplary variant HMGB1)	ctcgagatggactggacatggattctctttctagtggccgcagccacaagggtccactccatgggcaa aggagatcctaaaaagccgagaggcaaaatgtcctcatatgcattctttgtgcaaacttgccgggagg agcacaagaagaagcacccggatgctgcagtcaacttcgctgagttcgcaaagaagtgcgctgagagg tggaagaccatggcagctaaagaaaaggggaaatttgaagatatggcaaaggctgacaaggctcgtta tgaaagagaaatgaaaacctacatccccccccaaaggggagaccaaaaagaagttcaaggaccccaatg cacccaagaggcctccttcggccttcttcttgttctgttctgagtaccgccccaaaatcaaaggcgag catcctggcttatccattggtgatgttgcaaagaaactaggagagatgtggaacaacactgcagcaga tgacaagcagccctatgagaagaaagctgccaagctgaaggagaagtatgagaaggatattgctgcct acagagctaaaggaaaacctgatgcagcgaaaaaggggggtggtcaaggctgaaaaggcgaagaaaaag aaggaagaggaagatgatgaggaggatgaagaggatgaggaagaggaggaagaagaggaagacgaaga tgaagaagaagatgatgatgatgaa$X_1X_2X_3X_4X_5X_6X_7X_8X_9$ X_1= any nucleotide X_2= any nucleotide X_3= any nucleotide X_4= any nucleotide X_5= any nucleotide X_6= any nucleotide X_7= any nucleotide X_8= any nucleotide X_9= any nucleotide
SEQ ID NO: 2 (Nucleotide sequence coding for an exemplary variant HMGB1)	ctcgagatggactggacatggattctctttctagtggccgcagccacaagggtccactccatgggcaa aggagatcctaaaaagccgagaggcaaaatgtcctcatatgcattctttgtgcaaacttgccgggagg agcacaagaagaagcacccggatgctgcagtcaacttcgctgagttcgca
SEQ ID NO: 3 (Nucleotide sequence coding for an exemplary variant HMGB1)	gcagctaaagaaaaggggaaatttgaagatatggcaaaggctgacaaggctcgttatgaaagagaaat gaaaacctacatccccccccaaaggggagaccaaaaagaagttcaaggaccccaatgcacccaagaggc ctccttcggccttcttcttgttctgttctgagtaccgccccaaaatcaaaggcgagcatcctggctta tccattggtgatgttgcaaagaaactaggagagatgtggaacaacactgcagcagatgacaagcagcc ctatgagaagaaagctgccaagctgaaggagaagtatgagaaggatattgctgcctacagagctaaag gaaaacctgatgcagcgaaaaaggggggtggtcaaggctgaaaaggcgaagaaaaagaaggaagaggaa gatgatgaggaggatgaagaggatgaggaagaggaggaagaagaggaagacgaagatgaagaagaaga tgatgatgatgaa$X_1X_2X_3X_4X_5X_6X_7X_8X_9$ X_1= any nucleotide X_2= any nucleotide X_3= any nucleotide X_4= any nucleotide X_5= any nucleotide X_6= any nucleotide X_7= any nucleotide X_8= any nucleotide X_9= any nucleotide
SEQ ID NO: 4 (Amino acid sequence of an exemplary variant HMGB1)	LEMDWTWILFLVAAATRVHSMGKGDPKKPRGKMSSYAFFVQTCREEHKKK HPDAAVNFAEFAKKCAERWKTMAAKEKGKFEDMAKADKARYEREMKTYIP PKGETKKKEKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLG EMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEK AKKKKEEEDDEEDEEDEEEEEEEEDEDEEEDDDDEEAS
SEQ ID NO: 5 (Amino acid sequence of an exemplary variant HMGB1)	LEMDWTWILFLVAAATRVHSMGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDAAVNFAE FA
SEQ ID NO: 6 (Amino acid sequence of an exemplary	AAKEKGKFEDMAKADKARYEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGL SIGDVAKKLGEMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKAKKKKEEE DDEEDEEDEEEEEEEEDEDEEEDDDDEAS

variant HMGB1)	
SEQ ID NO: 7 (Nucleotide sequence coding for residues 89-108 of SEQ ID NO: 16)	tttaaagatccgaacgcgccgaaacgcccgccgagcgcgtttttttctgttttgcagcgaa
SEQ ID NO: 7 (Amino acid sequence of residues 89-108 of SEQ ID NO: 16)	FKDPNAPKRPPSAFFLFCSE
SEQ ID NO: 9 (Nucleotide sequence coding for residues 150-183 of SEQ ID NO: 16)	aaactgaaagaaaaatatgaaaaagatattgcggcgtatcgcgcgaaaggcaaaccggatgcggcgaa aaaaggcgtggtgaaagcggaaaaaagcaaaaaa
SEQ ID NO: 10 (Amino acid sequence of residues 150-183 of SEQ ID NO: 16)	KLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKSKK
SEQ ID NO: 11 (Exemplary coding sequence for human PH20)	ctgaactttcgcgcgccgccggtgattccgaacgtgccgtttctgtgggcgtggaacgcgccgagcga attttgcctgggcaaatttgatgaaccgctggatatgagcctgtttagctttattggcagcccgcgca ttaacgcgaccggccagggcgtgaccatttttttatgtggatcgcctgggctattatccgtatattgat agcattaccggcgtgaccgtgaacggcggcattccgcagaaaattagcctgcaggatcatctggataa agcgaaaaaagatattacctttttatatgccggtggataacctgggcatggcggtgattgattgggaag aatggcgcccgacctgggcgcgcaactggaaaccgaaagatgtgtataaaaaccgcagcattgaactg gtgcagcagcagaacgtgcagctgagcctgaccgaagcgaccgaaaaagcgaaacaggaatttgaaaa agcgggcaaagattttctggtggaaaccattaaactgggcaaactgctgcgcccgaaccatctgtggg gctattatctgtttccggattgctataaccatcattataaaaaaccgggctataacggcagctgcttt aacgtggaaattaaacgcaacgatgatctgagctggctgtggaacgaaagcaccgcgctgtatccgag catttatctgaacacccagcagagcccggtggcggcgaccctgtatgtgcgcaaccgcgtgcgcgaag cgattcgcgtgagcaaaattccggatgcgaaaagcccgctgccggtgtttgcgtatacccgcattgtg tttaccgatcaggtgctgaaatttctgagccaggatgaactggtgtatacctttggcgaaaccgtggc gctgggcgcgagcggcattgtgatttggggcaccctgagcattatgcgcagcatgaaaagctgcctgc tgctggataactatatggaaaccattctgaacccgtatattattaacgtgaccctggcggcgaaaatg tgcagccaggtgctgtgccaggaacagggcgtgtgcattcgcaaaaactggaacagcagcgattatct gcatctgaacccggataactttgcgattcagctggaaaaaggcggcaaagaaaccgtgcgcggcaaac cgaccctggaagatctggaacagtttagcgaaaaattttattgcagctgctatagcaccctgagctgc aaagaaaaagcggatgtgaaagataccgatgcggtggatgtgtgcattgcggatggcgtgtgcattga tgcgtttctgaaaccgccgatggaaaccgaagaaccgcagatttttttat
SEQ ID NO: 12 (Amino acid sequence human PH20) NCBI Reference Sequence: NP_694859.1	LNFRAPPVIP NVPFLWAWNA PSEFCLGKFD EPLDMSLFSF IGSPRINATG QGVTIFYVDR LGYYPYIDSI TGVTVNGGIP QKISLQDHLD KAKKDITFYM PVDNLGMAVI DWEEWRPTWA RNWKPKDVYK NRSIELVQQQ NVQLSLTEAT EKAKQEFEKA GKDFLVETIK LGKLLRPNHL WGYYLFPDCY NHHYKKPGYN GSCFNVEIKR NDDLSWLWNE STALYPSIYL NTQQSPVAAT LYVRNRVREA IRVSKIPDAK SPLPVFAYTR IVFTDQVLKF LSQDELVYTF GETVALGASG IVIWGTLSIM RSMKSCLLLD NYMETILNPY IINVTLAAKM CSQVLCQEQG VCIRKNWNSS DYLHLNPDNF AIQLEKGGKE TVRGKPTLED LEQFSEKFYC SCYSTLSCKE KADVKDTDAV DVCIADGVCI DAFLKPPMET EEPQIFY
SEQ ID NO: 13 (Nucleotide sequence coding for an exemplary variant HMGB1)	ctcgagatggactggacatggattctctttctagtggccgcagccacaagggtccactccatgggcaa aggagatcctaaaaagccgagaggcaaaatgtcctcatatgcattctttgtgcaaacttgccgggagg agcacaagaagaagcacccggatgctgcagtcaacttcgctgagttcgcaaagaagtgcgctgagagg tggaagaccatggcagctaaagaaaaggggaaatttgaagatatggcaaaggctgacaaggctcgtta tgaaagagaaatgaaaacctacatccccccccaaaggggagaccaaaaagaagttcaaggaccccaatg cacccaagaggcctccttcggccttcttcttgttctgttctgagtaccgccccaaaatcaaaggcgag catcctggcttatccattggtgatgttgcaaagaaactaggagagatgtggaacaacactgcagcaga

	tgacaagcagccctatgagaagaaagctgccaagctgaaggagaagtatgagaaggatattgctgcct acagagctaaaggaaaacctgatgcagcgaaaaaggggtggtcaaggctgaaaaggcgaagaaaaag aaggaagaggaagatgatgaggaggatgaagaggatgaggaagaggaggaagaagaggaagacgaaga tgaagaagaagatgatgatgatgaa
SEQ ID NO: 14 (Nucleotide sequence coding for an exemplary variant HMGB1)	Ctcgagatggactggacatggattctctttctagtggccgcagccacaagggtccactccatgggcaa aggagatcctaaaaagccgagaggcaaaatgtcctcatatgcattctttgtgcaaacttgccgggagg agcacaagaagaagcacccggatgctgcagtcaacttcgctgagttcgcaaagaagtgcgctgagagg tggaagaccatggcagctaaagaaaaggggaaatttgaagatatggcaaaggctgacaaggctcgtta tgaaagagaaatgaaaacctacatccccccccaaaggggagaccaaaaagaagttcaaggaccccaatg cacccaagaggcctccttcggccttcttcttgttctgttctgagtaccgccccaaaatcaaaggcgag catcctggcttatccattggtgatgttgcaaagaaactaggagagatgtggaacaacactgcagcaga tgacaagcagccctatgagaagaaagctgccaagctgaaggagaagtatgagaaggatattgctgcct acagagctaaaggaaaacctgatgcagcgaaaaaggggtggtcaaggctgaaaaggcgaagaaaaag aaggaagaggaagatgatgaggaggatgaagaggatgaggaagaggaggaagaagaggaagacgaaga tgaagaagaagatgatgatgaa$X_1X_2X_3$gctagc X_1= any nucleotide X_2= any nucleotide X_3= any nucleotide
SEQ ID NO: 15 (Amino acid sequence of an exemplary variant HMGB1)	LEMDWTWILFLVAAATRVHSMGKGDPKKPRGKMSSYAFFVQTCREEHKKK HPDAAVNFAEFAKKCAERWKTMAAKEKGKFEDMAKADKARYEREMKTYIP PKGETKKKEKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLG EMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEK AKKKKEEEDDEEDEEDEEEEEEEEDEDEEEDDDDE
SEQ ID NO: 16 (Amino acid sequence of human HMGB1 wt) Genbank Accession No: GenBank: AAP36330.1	MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADK ARYEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNT AADDKQPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKSKKKKEEEEDEEDEEDEEEEEDEE DEDEEEDDDDE
SEQ ID NO: 17 (Amino acid sequence of an exemplary variant HMGB1)	LEMDWTWILFLVAAATRVHSMGKGDPKKPRGKMSSYAFFVQTCREEHKKK HPDAAVNFAEFAKKCAERWKTMAAKEKGKFEDMAKADKARYEREMKTYIP PKGETKKKEKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLG EMWNNTAADDKQPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEK AKKKKEEEDDEEDEEDEEEEEEEEDEDEEEDDDDE*AS * can be any amino acid
SEQ ID NO: 18 (Amino acid sequence human IgE) Uniprot Accession No: P01854	ASTQSPSVFPLTRCCKNIPSNATSVTLGCLATGYFPEPVMVTWDTGSLNGTTMTLPATTLTLSGHYAT ISLLTVSGAWAKQMFTCRVAHTPSSTDWVDNKTFSVCSRDFTPPTVKILQSSCDGGGHFPPTIQLLCL VSGYTPGTINITWLEDGQVMDVDLSTASTTQEGELASTQSELTLSQKHWLSDRTYTCQVTYQGHTFED STKKCADSNPRGVSAYLSRPSPFDLFIRKSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEK QRNGTLTVTSTLPVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAFATPEWPGSRDK RTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTKGSGFFVFSRLEVTRAEWEQKDEFICRAV HEAASPSQTVQRAVSVNPGK
SEQ ID NO: 19 (Nucleotide sequence coding for an exemplary variant HMGB1)	Ctcgagatggactggacatggattctctttctagtggccgcagccacaagggtccactccatgggcaa aggagatcctaaaaagccgagaggcaaaatgtcctcatatgcattctttgtgcaaacttgccgggagg agcacaagaagaagcacccggatgctgcagtcaacttcgctgagttcgcaaagaagtgcgctgagagg tggaagaccatggcagctaaagaaaaggggaaatttgaagatatggcaaaggctgacaaggctcgtta tgaaagagaaatgaaaacctacatccccccccaaaggggagaccaaaaagaagttcaaggaccccaatg cacccaagaggcctccttcggccttcttcttgttctgttctgagtaccgccccaaaatcaaaggcgag catcctggcttatccattggtgatgttgcaaagaaactaggagagatgtggaacaacactgcagcaga tgacaagcagccctatgagaagaaagctgccaagctgaaggagaagtatgagaaggatattgctgcct acagagctaaaggaaaacctgatgcagcgaaaaaggggtggtcaaggctgaaaaggcgaagaaaaag aaggaagaggaagatgatgaggaggatgaagaggatgaggaagaggaggaagaagaggaagacgaaga tgaagaagaagatgatgatgatgaagaagctagc
SEQ ID NO: 20 First nuclear location signal (NLS1) of an exemplary	KKKHPDAAVNFAEFAKKCAERWKTMAA

variant HMGB1	
SEQ ID NO: 21 Second nuclear location signal (NLS2) of an exemplary variant HMGB1	EKAKKKK
SEQ ID NO: 22 (Amino acid sequence of an exemplary variant HMGB1)	MGKGDPKKPR GKMSSYAFFV QT**C**REEHKKK HPDAAVNFAE FAKK**C**AERWK TMAAKEKGKF EDMAKADKAR YEREMKTYIP PKGETKKKEK DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKAAK LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK AKKKKEEEDD EEDEEDEEEE EEEEDEDEEE DDDDE
SEQ ID NO: 23 (Amino acid sequence of an exemplary variant HMGB1)	MGKGDPKKPR GKMSSYAFFV QT**C**REEHKKK HPDAAVNFAE FAKK**C**AERWK TMAAKEKGKF EDMAKADKAR YEREMKTYIP PKGETKKKEK DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKAAK LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK AKKKKEEEDD EEDEEDEEEE EEEEDEDEEE DDDDE*AS * can be any amino acid
SEQ ID NO: 24 (Amino acid sequence of an exemplary variant HMGB1)	MDWTWILFLV AAATRVHSMG KGDPKKPRG KMSSYAFFV QT**C**REEHKKK HPDAAVNFAE FAKK**C**AERWK TMAAKEKGKF EDMAKADKAR YEREMKTYIP PKGETKKKEK DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKAAK LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK AKKKKEEEDD EEDEEDEEEE EEEEDEDEEE DDDDE
SEQ ID NO: 25 (Amino acid sequence of an exemplary variant HMGB1)	MDWTWILFLV AAATRVHSMG KGDPKKPRG KMSSYAFFV QT**C**REEHKKK HPDAAVNFAE FAKK**C**AERWK TMAAKEKGKF EDMAKADKAR YEREMKTYIP PKGETKKKEK DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKAAK LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK AKKKKEEEDD EEDEEDEEEE EEEEDEDEEE DDDDE*AS * can be any amino acid

WHAT IS CLAIMED IS:

1. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 13, or a fragment thereof.

2. The oncolytic vaccinia virus of claim 1, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 13, or a fragment thereof.

3. The oncolytic vaccinia virus of claim 1 or 2, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof.

4. The oncolytic vaccinia virus of any one of claims 1-3, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15.

5. The oncolytic vaccinia virus of any one of claims 1-3, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15.

6. The oncolytic vaccinia virus of any one of claims 1-5, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 15.

7. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises a mutation in its first (NLS1), the second nuclear localization signal (NLS2), or any combinations thereof.

8. The oncolytic vaccinia virus of claim 7, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 further comprises a mutation in boxA, boxB, or any combinations thereof.

9. The oncolytic vaccinia virus of claim 7 or 8, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof.

10. The oncolytic vaccinia virus of claim 9, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the HMGB1 variant comprises cysteine residues at positions 43 and 65 of SEQ ID NO: 15.

11. The oncolytic vaccinia virus of claim 9 or 10, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the HMGB1 variant comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 15.

12. The oncolytic vaccinia virus of claim 9 or 10, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein said HMGB1 variant comprises residues 21-74, and 109-128 or 170-203 of SEQ ID NO: 15.

13. The oncolytic vaccinia virus of any one of claims 7-12, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 15.

14. The oncolytic vaccinia virus of any one of claims 7-13, comprising the exogenous nucleic acid sequence that codes for the variant HMGB1, wherein said nucleic acid sequence is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% to SEQ ID NO: 13, or a fragment thereof.

15. The oncolytic vaccinia virus of any one of claims 7-14, further comprising an exogenous nucleic acid sequence that codes for an antibody or an antigen binding fragment thereof operably linked to the exogenous nucleic acid sequence that codes for the variant HMGB1.

16. The oncolytic vaccinia virus of any one of claims 7-15, further comprising an exogenous nucleic acid sequence that codes for a hyaluronidase operably linked to the exogenous nucleic acid sequence that codes for the variant HMGB1.

17. The oncolytic vaccinia virus of any one of claims 7-16, comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutation in NLS1, wherein the mutation in NLS1 promotes cytoplasmic re-location of the variant HMGB1.

18. The oncolytic vaccinia virus of any one of claims 7-16, comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutation in NLS2, wherein the mutation in NLS2 promotes cytoplasmic re-location of the variant HMGB1.

19. The oncolytic vaccinia virus of any one of claims 7-16, comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutations in NLS1 and NLS2, wherein the mutations in NLS1 and NLS2 promote cytoplasmic re-location of the variant HMGB1.

20. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 is less than or equal to 95% homologous to human HMGB1 (SEQ ID NO: 16).

21. The oncolytic vaccinia virus of claim 20, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 1-85, 89-108, and 150-183 of SEQ ID NO: 16.

22. The oncolytic vaccinia virus of claim 20 or 21, wherein the variant HMGB1 comprises residues 1-85 of SEQ ID NO: 16.

23. The oncolytic vaccinia virus of claim 20 or 21, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 89-108 of SEQ ID NO: 16.

24. The oncolytic vaccinia virus of claim 20 or 21, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 150-183 of SEQ ID NO: 16.

25. The oncolytic vaccinia virus of any one claims 20-24, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 1-85, 89-108, and 150-183 of SEQ ID NO: 16.

26. The oncolytic vaccinia virus of any one of claims 20-25, comprising the exogenous nucleic acid that codes for the variant HMGB1wherein the variant HMGB1 comprises mutations at one or more of residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

27. The oncolytic vaccinia virus of any one of claims 20-25, comprising the exogenous nucleic acid that codes for the variant HMGB1wherein the variant HMGB1 comprises mutations at residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

28. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises residues 23, 45, 89-108, and 150-183 of SEQ ID NO: 16, and wherein the variant HMGB1 comprises an amino acid sequence that is less than or equal to 95% homologous to SEQ ID NO: 16.

29. The oncolytic vaccinia virus of claim 28, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 23-45, 89-108, 150-183 of SEQ ID NO: 16.

30. The oncolytic vaccinia virus of claim 28 or 29, wherein the variant HMGB1 comprises mutations at one or more of residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

31. The oncolytic vaccinia virus of claim 28 or 29, wherein the variant HMGB1 comprises mutations at residues 35, 42, 89, 181, 189, and 202 of SEQ ID NO: 16.

32. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 14, or a fragment thereof.

33. The oncolytic vaccinia virus of claim 32, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 14, or the fragment thereof.

34. The oncolytic vaccinia virus of claim 31 or 32, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 17, or a fragment thereof.

35. The oncolytic vaccinia virus of any one of claims 32-34, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17.

36. The oncolytic vaccinia virus of any one of claims 32-34, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17.

37. The oncolytic vaccinia virus of any one of claims 32-36, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 17.

38. An oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 19, or a fragment thereof.

39. The oncolytic vaccinia virus of claim 38, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 14, or the fragment thereof.

40. The oncolytic vaccinia virus of claim 38 or 39, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 17, or a fragment thereof.

41. The oncolytic vaccinia virus of any one of claims 38-40, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17.

42. The oncolytic vaccinia virus of any one of claims 38-40, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 comprises residues 21-74, 109-128, and 170-203 of SEQ ID NO: 17.

43. The oncolytic vaccinia virus of any one of claims 38-42, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the variant HMGB1 does not comprise residues 1-20 of SEQ ID NO: 17.

44. The oncolytic vaccinia virus of any one of claims 1-43, comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutation in NLS2, wherein the mutation in NLS2 promotes cytoplasmic re-location of the variant HMGB1.

45. The oncolytic vaccinia virus of any one of claims 1-44, comprising the exogenous nucleic acid that codes for the variant HMGB1 comprising the mutations in NLS1 and NLS2, wherein the mutations in NLS1 and NLS2 promotes cytoplasmic re-location of the variant HMGB1.

46. The oncolytic vaccinia virus of any one of claims 1-45, wherein the variant HMGB1 expressed from the vaccinia virus is secreted into the cytoplasm.

47. The oncolytic vaccinia virus of any one of claims 1-46, wherein expression of the variant HMGB1 from the vaccinia virus enhances cellular autophagy, compared to an otherwise identical vaccinia virus that lacks the exogenous nucleic acid coding for the variant HMGB1.

48. The oncolytic vaccinia virus of any one of claims 1-47, wherein expression of the variant HMGB1 enhances tumor-specific replication of the vaccinia virus, compared to an otherwise identical vaccinia virus that lacks the exogenous nucleic acid coding for the variant HMGB1.

49. The oncolytic vaccinia virus of any one of claims 1-48, wherein expression of the variant HMGB1 enhances cytotoxic immune response against a tumor cell infected by the vaccinia virus, compared to an otherwise identical vaccinia virus that lacks the exogenous nucleic acid coding for the variant HMGB1

50. The oncolytic vaccinia virus of any one of claims 1-49, wherein expression of the variant HMGB1 induces cytotoxic immune response against a tumor cell in the vicinity of a tumor cell infected by the vaccinia virus, wherein the tumor cell in the vicinity is not infected by the vaccinia virus.

51. The oncolytic vaccinia virus of any one of claims 1-50, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the vaccinia virus comprises one or more deletions in its genome.

52. The oncolytic vaccinia virus of any one of claims 1-51, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the vaccinia virus comprises a thymidine kinase gene deletion and the exogenous nucleic acid is inserted into the thymidine kinase gene locus.

53. The oncolytic vaccinia virus of any one of claims 1-51, comprising the exogenous nucleic acid that codes for the variant HMGB1, wherein the vaccinia virus comprises deletions of one or more virulence genes.

54. The oncolytic vaccinia virus of any one of claims 1-53, wherein the virus is an extracellular enveloped virus (EEV).

55. The oncolytic vaccinia virus of any one of claims 1-54, wherein the vaccinia virus further expresses a hyaluronidase enzyme.

56. The oncolytic vaccinia virus of any one of claims 1-55, wherein the vaccinia virus expresses the hyaluronidase enzyme, and wherein the hyaluronidase enzyme increases the spread of the vaccinia virus in a tumor microenvironment, compared to the spread of an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid coding for the hyaluronidase enzyme operable linked to the exogenous nucleic acid coding for the variant HMGB1.

57. The oncolytic vaccinia virus of any one of claims 1-56, wherein the vaccinia virus expresses the hyaluronidase enzyme, and wherein the hyaluronidase enzyme increases the spread of the vaccinia virus in a tumor microenvironment.

58. The oncolytic vaccinia virus of any one of claims 1-57, wherein the vaccinia virus further expresses a protein that stabilizes the variant HMGB1 in circulation.

59. The oncolytic vaccinia virus of claim 58, wherein the protein that stabilizes the variant HMGB1 in circulation is an immunoglobulin or a domain thereof.

60. The oncolytic vaccinia virus of claim 59, wherein the immunoglobulin is IgE.

61. The oncolytic vaccinia virus of claim 59, wherein the immunoglobulin domain is an Fc domain.

62. The oncolytic vaccinia virus of claim 60, comprising the exogenous nucleic acid sequence that codes for the variant HMGB1, wherein the further exogenous nucleic acid sequence coding for the IgE is fused upstream to the variant HMGB1 coding sequence.

63. The oncolytic vaccinia virus of any one of claims 1-62, wherein the virus further expresses an efflux pump blocker.

64. The oncolytic vaccinia virus of any one of claims 1-63, wherein the virus further expresses a chemotherapy sensitizer.

65. The oncolytic vaccinia virus of any one of claims 1-64, wherein the virus comprises a modification that enhances sensitization to chemotherapy.

66. The oncolytic vaccinia virus of any one of claims 1-65, wherein the virus comprises a modification that attracts chimeric antigen receptor T cells.

67. The oncolytic vaccinia virus of any one of claims 1-66, wherein the virus enhances efficacy of a CAR T cell based therapy.

68. An isolated polynucleotide that codes for a variant HMGB1, wherein the polynucleotide comprises a sequence that is at least 85% homologous to SEQ ID NO: 13, or a fragment thereof.

69. The isolated polynucleotide of claim 68, wherein the polynucleotide codes for a variant HMGB1, wherein the variant HMGB1 comprises an amino acid sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 15, or a fragment thereof.

70. The isolated polynucleotide of any one of claims 68 or 69, coding for the variant HMGB1, wherein the variant HMGB1 comprises a mutation in its first (NLS1) or second nuclear localization signal (NLS2).

71. The isolated polynucleotide of claim 70, coding for the variant HMGB1, wherein the variant HMGB1 further comprises a mutation in boxA or boxB.

72. The isolated polynucleotide of claim 70, coding for the variant HMGB1, wherein the variant HMGB1 further comprises mutations in boxA or boxB.

73. The isolated polynucleotide any one of claims 68-72, wherein the variant HMGB1 comprises cysteine residues at positions 43 and 65 of SEQ ID NO: 15.

74. The isolated polynucleotide of any one of claims 68-73, wherein said HMGB1 variant comprises at least one of residues 21-74, 109-128, and 170-183 of SEQ ID NO: 15.

75. The isolated polynucleotide of any one of claims 68-73, wherein said HMGB1 variant comprises residues 21-105, 108-182, and 183-205 of SEQ ID NO: 15.

76. A pharmaceutical composition comprising an oncolytic vaccinia virus as defined in any one of claims 1-67 or an isolated polynucleotide as defined in any one of claims 68-75, a solubilizing agent, and an excipient.

77. The pharmaceutical composition of claim 76, 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 combination thereof.

78. The pharmaceutical composition of claim 77, wherein the excipient comprises di-sodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, myo-inositol, sorbitol, or any combination thereof.

79. The pharmaceutical composition of any one of claims 76-78, that does not comprise a preservative.

80. The pharmaceutical composition of any one of claims 76-79, further comprising one or more of a preservative, a diluent, and a carrier.

81. The pharmaceutical composition of any one of claims 76-80, further comprising an additional active ingredient or a salt thereof.

82. The pharmaceutical composition of any one of claims 76-81, wherein the solubilizing agent is sterile water.

83. The pharmaceutical composition of any one of claims 76-81, comprising an additional active ingredient, wherein the additional active ingredient is a further oncolytic virus.

84. A process for producing an oncolytic vaccinia virus as defined in any one of claims 1-67, the process comprising: (i) generating a modified vaccinia virus DNA vector by operably linking a vaccinia virus base nucleic acid sequence to the exogenous nucleic acid sequence according to any one of claims 1-67; (ii) transfecting mammalian cells with the modified vaccinia virus DNA vector; (iii) culturing the mammalian cells in conditions suitable for viral replication; and (iv) harvesting the viral particles.

85. The process of claim 84, wherein the mammalian cells comprise HeLa cells, 293 cells, or Vero cells.

86. The process of claim 84 or 85, wherein the exogenous nucleic acid, according to any one of claims 1-67, in the modified vaccinia virus DNA vector promotes a population of viral particles predominantly containing extracellular enveloped viruses (EEV).

87. The process of any one of claims 84-86, wherein the exogenous nucleic acid, according to any one of claims 1-67, in the modified vaccinia virus DNA vector results in a higher titer in at least one of HeLa cells and 293 cells, compared to an otherwise identical vaccinia virus DNA vector which does not comprise the exogenous nucleic acid according to any one of claims 1-67.

88. A method of treating a cancer, comprising administering to a subject a therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83.

89. The method of claim 88, comprising the administration of the therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein the cancer is a solid tumor, a leukemia, or a lymphoma.

90. A method of treating a tumor, comprising administering to a subject a therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83.

91. The method of claim 87, comprising the administration of the therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein the tumor is a solid tumor, a leukemia, or a lymphoma.

92. The method of any one of claims 84-88, comprising administering to the subject in need thereof the a therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83.

93. A method of treating a tumor comprising high bioavailability of fatty acids, comprising administering an oncolytic vaccinia virus as defined in any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein the vaccinia virus demonstrates increased tumor selective replication in the tumor comprising the high bioavailability of free fatty acids.

94. A method of treating an obese cancer patient, comprising administering an oncolytic vaccinia virus as defined in any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein the vaccinia virus demonstrates increased tumor selective replication in the obese cancer patient.

95. The method of claim 93 or 94, wherein the increased tumor selective replication is relative to a tumor selective replication of an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid sequence coding for the variant HMGB1.

96. The method of claim 94, wherein the increased tumor selective replication is relative to the tumor selective replication of the vaccinia virus in a non-obese cancer patient.

97. The method of any one of claims 94-96, wherein the vaccinia virus produces an increased proportion of EEV in the obese cancer patient.

98. The method of claim 97, wherein the increased proportion of EEV in the obese cancer patient is relative to the proportion of EEV produced by an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid sequence coding for the variant HMGB1.

99. The method of claim 98, wherein the increased proportion of EEV in the obese cancer patient is relative to the proportion of EEV produced by the vaccinia virus in a non-obese cancer patient.

100. A method of reducing a likelihood of cancer relapse after chemotherapy, comprising administering an oncolytic vaccinia virus as defined in any one of claims 1-67, or a pharmaceutical formulation as defined in any one of claims 76-83.

101. A method of treatment, comprising administering an oncolytic vaccinia virus as defined in any one of claims 1-67, or a pharmaceutical formulation as defined in any one of claims 76-83 to a subject who has failed a prior treatment comprising an immunotherapy.

102. A method of treatment, comprising administering an oncolytic vaccinia virus as defined in any one of claims 1-67, or a pharmaceutical formulation as defined in any one of claims 76-83 to a subject who has relapse of cancer after chemotherapy.

103. A method of treatment, comprising administering an oncolytic vaccinia virus as defined in any one of claims 1-67, or a pharmaceutical formulation as defined in any one of claims 76-83 to a subject who has a peritoneal cancer.

104. The method of claim any one of claims 101-103, wherein the subject is obese.

105. The method of any one of claims 88-104, comprising administration of an oncolytic vaccinia virus as defined in any one of claims 1-67, or a pharmaceutical formulation as defined in any one of claims 76-83, wherein the method further comprises administration of a further therapy.

106. The method of any one of claims 88-105, comprising administration of the further therapy in combination with an oncolytic vaccinia virus as defined in any one of claims 1-67 or a pharmaceutical formulation as defined in any one of claims 76-83, 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.

107. The method of claim 106, wherein the further therapy comprises chemotherapy.

108. The method of claim 107, wherein the combination of administration of an oncolytic vaccinia virus as defined in any one of claims 1-67 or a pharmaceutical formulation as defined in any one of claims 76-83, and chemotherapy, results in a synergistic effect, compared to a combination of the chemotherapy and an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid that codes for a variant HMGB1 as defined in any one of claims 1-67.

109. The method of any one of claims 106-108, wherein the chemotherapy in combination with an oncolytic vaccinia virus as defined in any one of claims 1-67 or a pharmaceutical formulation as defined in any one of claims 76-83 is administered at a lower dose than a chemotherapy in combination with an otherwise identical vaccinia virus that does not comprise the exogenous nucleic acid that codes for a variant HMGB1 as defined in any one of claims 1-67.

110. The method of any one of claims 105-109, comprising administration of the further therapy, wherein the further therapy is administered concurrently or sequentially.

111. The method of claim 110, comprising sequential administration of the further therapy, wherein the further therapy is administered prior to administering an oncolytic vaccinia virus according to any one of claims 1-67, or a pharmaceutical composition according to any one of claims 76-83.

112. The method of claim 111, comprising sequential administration of the further therapy, wherein the further therapy is administered after administering an oncolytic vaccinia virus according to any one of claims 1-67, or a pharmaceutical composition according to any one of claims 76-83.

113. A method of producing a toxic effect in cancer cells, the method comprising administering to a population of cancer cells a therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67, or a pharmaceutical composition according to any one of claims 76-83.

114. The method of claim 113, comprising the administration of the therapeutically effective amount of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein not every cancer cell in the population of cancer cells is infected with the oncolytic vaccinia virus.

115. The method of claim 114, wherein growth of a non-infected cancer cell is inhibited without direct infection of the oncolytic vaccinia virus.

116. The method of claim 114 or 115, wherein cytotoxic immune response in a non-infected cancer cell is induced without direct infection by the oncolytic vaccinia virus.

117. A method for drug delivery comprising introducing into a tumor of a subject an oncolytic vaccinia vector as defined in any of claims 1-67 or a pharmaceutical formulation as defined in any one of claims 76-83.

118. The method of claim 117, wherein the drug comprises a nanoparticle conjugated to a tumor receptor ligand.

119. A method for treating cancer in a subject, comprising administering cells infected with an oncolytic vaccinia virus as defined in any one of claims 1-67.

120. The method of claim 119, wherein the method increases efficacy of oncolytic vaccinia virus based cancer therapy.

121. The method of any one of claims 88-120, comprising the administration of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein 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.

122. The method of any one of claims 88-121, comprising the administration of an oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to

any one of claims 76-83, wherein the oncolytic vaccinia virus or the pharmaceutical composition is administered at a dosage that comprises about 5 x 10^9 PFU/mL of the oncolytic vaccinia virus.

123. The method of any one of claims 88-122, comprising the administration of the oncolytic vaccinia virus according to any one of claims 1-67 or a pharmaceutical composition according to any one of claims 76-83, wherein 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.

124. The method of claim 123, 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.

125. The method of claim 123 or 124, wherein the first, second, and third periods of time are each from about 1 week to about 3 weeks.

126. The method of any one of claims 88-125, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83, wherein 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.

127. The method of any one of claims 88-126, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83, wherein the oncolytic vaccinia virus or the pharmaceutical composition is administered in a liquid dosage form, a solid dosage form, 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 .

128. The method of any one of claims 88-127, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83, wherein 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.

129. The method of any one of claims 88-128, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according

to any one of claims 76-83, wherein 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.

130. The method of any one of claims 88-129, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83, wherein the oncolytic vaccinia virus or the pharmaceutical composition is administered intravenously, intraperitoneally, or by an intratumoral injection.

131. The method of any one of claims 88-130, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83, wherein the vaccinia virus or the pharmaceutical formulation is administered as a bolus injection or a slow infusion.

132. The method of any one of claims 88-131, comprising administering the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83, wherein the administration of 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.

133. The method of any one of claims 88-132, 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.

134. The method of any one of claims 88-133, 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.

135. The method of any one of claims 88-134, 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, in a liposomal formulation, a dosage form comprising nanoparticles, a dosage form comprising microparticles, a polymeric dosage form, or any combinations thereof .

136. The method of any one of claims 88-135, comprising administration of the further therapy, wherein the further therapy is administered orally, intravenously, by an intratumoral injection, or by radiation.

137. The method of any one of claims 88-136, comprising the administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83 to a subject in need thereof, wherein the subject is human.

138. The method of any one of claims 88-137, comprising the administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83 to the subject in need thereof, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition the subject has been diagnosed with a cancer or a tumor.

139. The method of any one of claims 88-138, comprising the administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83 to the subject in need thereof, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition the subject is diagnosed with a cancer or a tumor, and wherein the subject is obese.

140. The method of any one of claims 88-139, comprising the administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83 to the subject in need thereof in combination with the further therapy, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition or the further therapy the subject has been diagnosed with a cancer or a tumor.

141. The method of any one of claims 88-140, comprising the administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83 to the subject in need thereof in combination with the further therapy, wherein prior to administration of the oncolytic vaccinia virus or the pharmaceutical composition the subject is diagnosed with a cancer or a tumor, and wherein the subject is obese.

142. The method of any one of claims 88-141, wherein the subject is administered a ketogenic diet prior to, concurrently, or following administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83.

143. The method of any one of claims 88-142, wherein the subject is on short term fasting prior to, concurrently, or following administration of the oncolytic vaccinia virus according to any one of claims 1-67, or the pharmaceutical composition according to any one of claims 76-83.

144. A modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 13, or a fragment thereof.

145. A modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises a mutation in its first nuclear localization signal (NLS1), second nuclear localization signal (NLS2), or any combinations thereof.

146. A modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 is less than or equal to 95% homologous to human HMGB1 (SEQ ID NO: 16).

147. A modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the variant HMGB1 comprises at least one of residues 23, 45, 89-108, and 150-183 of SEQ ID NO: 16, and wherein the variant HMGB1 comprises an amino acid sequence that is less than or equal to 95% homologous to SEQ ID NO: 16.

148. A modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 14, or a fragment thereof.

149. A modified virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 80% homologous to SEQ ID NO: 19, or a fragment thereof.

150. The modified virus according to any one of claims 144-149, comprising Herpes Simplex Virus (HSV), Adenovirus, Polio virus, VSV, Coxsackievirus, Reovirus, Lentivirus, Adeno-associated virus (AAV), Measles virus, Maraba virus, Newcastle disease (NDV), Seneca valley virus, Mengovirus, or Myxomavir.

151. The oncolytic vaccinia virus of any one of claims 7-19, wherein NLS1 comprises SEQ ID NO: 20 and NLS2 comprise SEQ ID NO: 21.

FIGURE 1

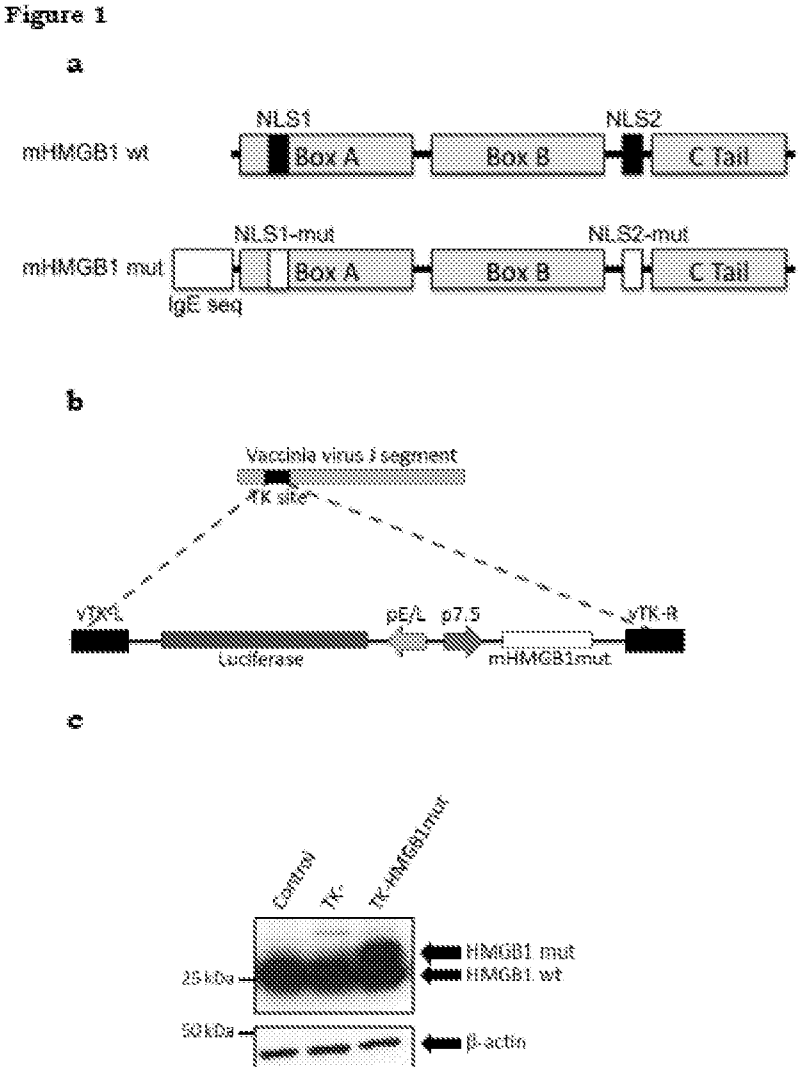


Figure 1
a
mHMGB1 wt
NLS1
NLS2
Box A
Box B
C Tail
mHMGB1 mut
NLS1-mut
NLS2-mut
IgE seq
b
Vaccinia virus J segment
TK site
vTK-L
pE/L
p7.5
vTK-R
Luciferase
mHMGB1mut
c
Control
TK-
TK-HMGB1mut
25 kDa
50 kDa
HMGB1 mut
HMGB1 wt
β-actin

Cont'd. FIGURE 1

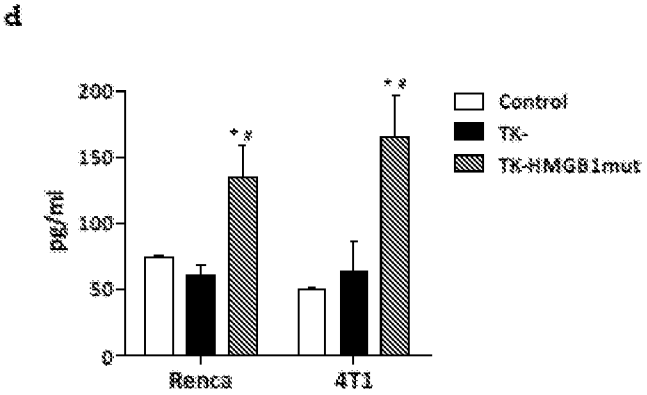
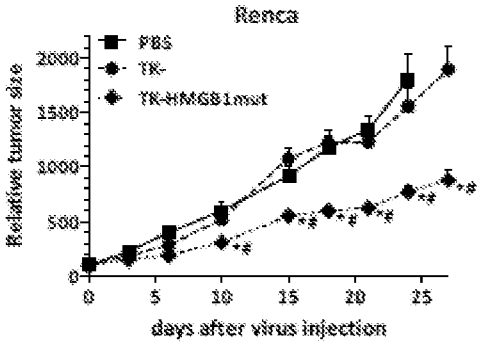


FIGURE 2

Figure 2

a



b

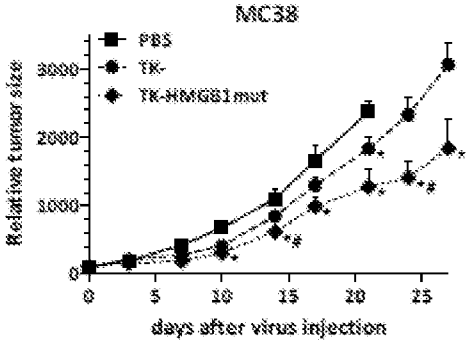
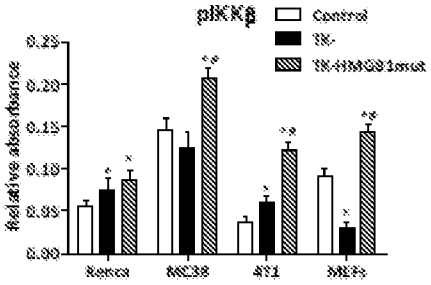


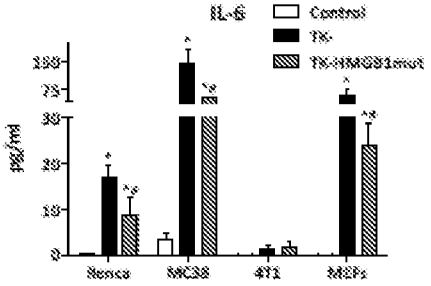
FIGURE 3

Figure 3

a



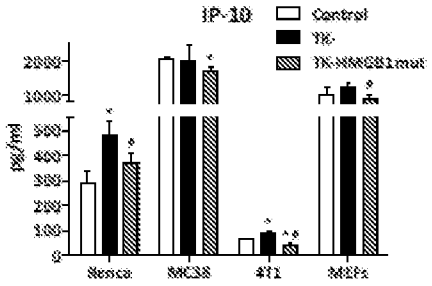
b



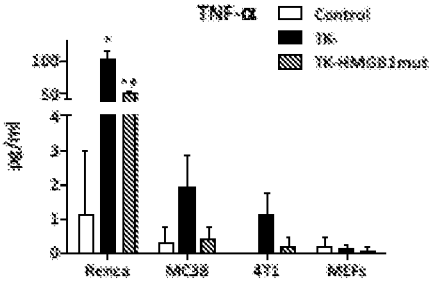
Cont'd FIGURE 3

Figure 3 – cont'd.

c



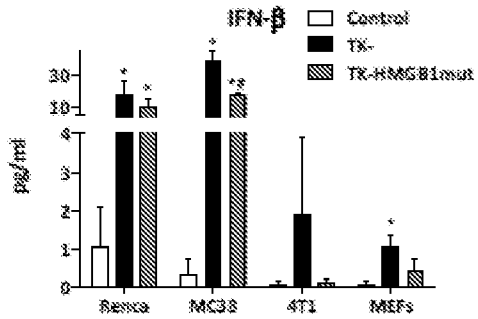
d



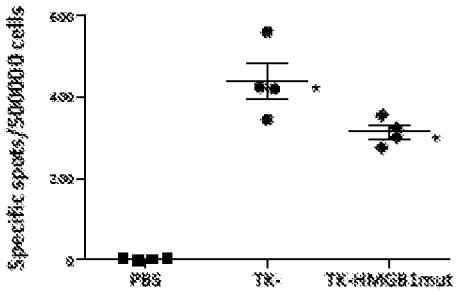
Cont'd. FIGURE 3

Figure 3 – cont'd.

e



f



Cont'd. FIGURE 3

Figure 3 – cont'd.

g

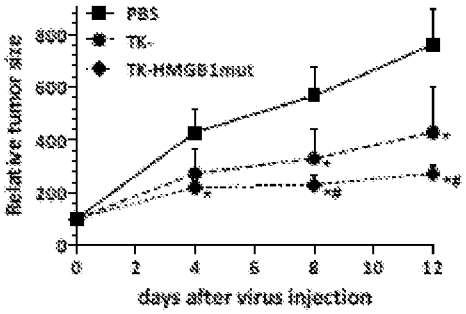


FIGURE 4

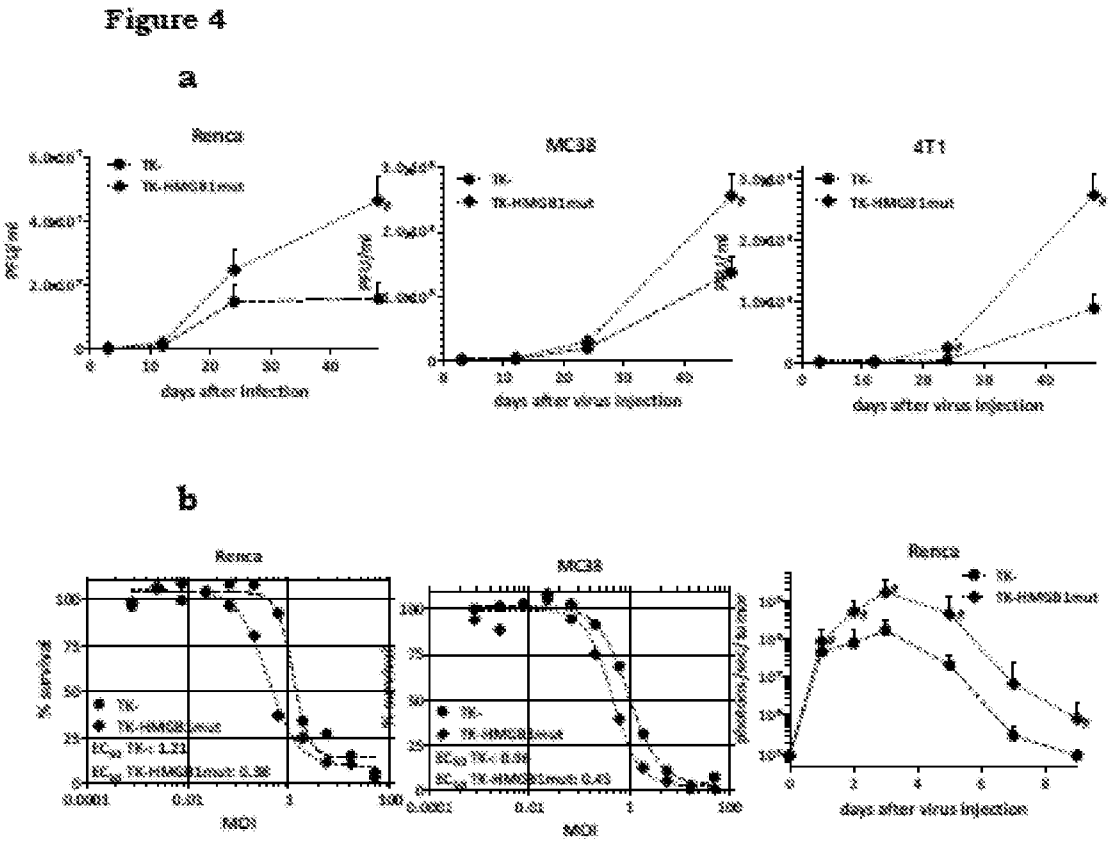


Figure 4
a
Renca
MC38
4T1
days after infection
days after virus injection
b
Renca
MC38
Renca
MOI
days after virus injection

Cont'd FIGURE 4

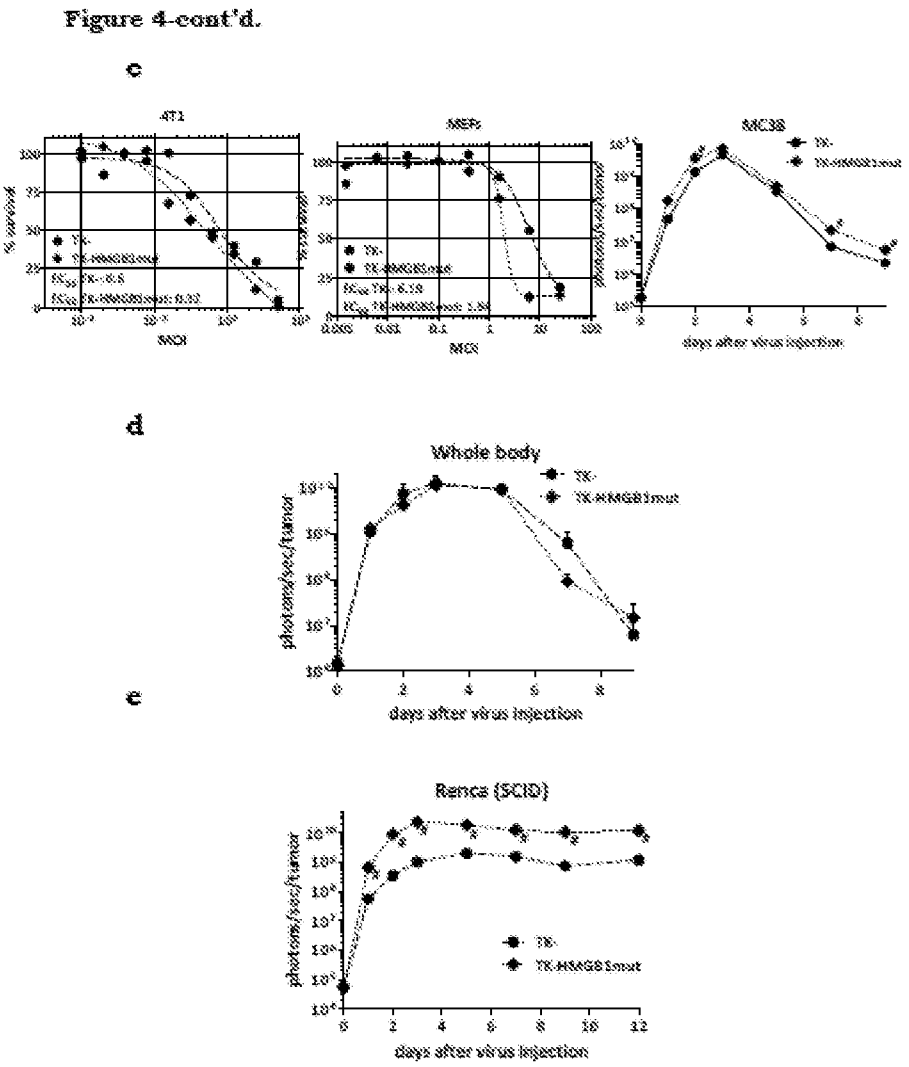


FIGURE 5

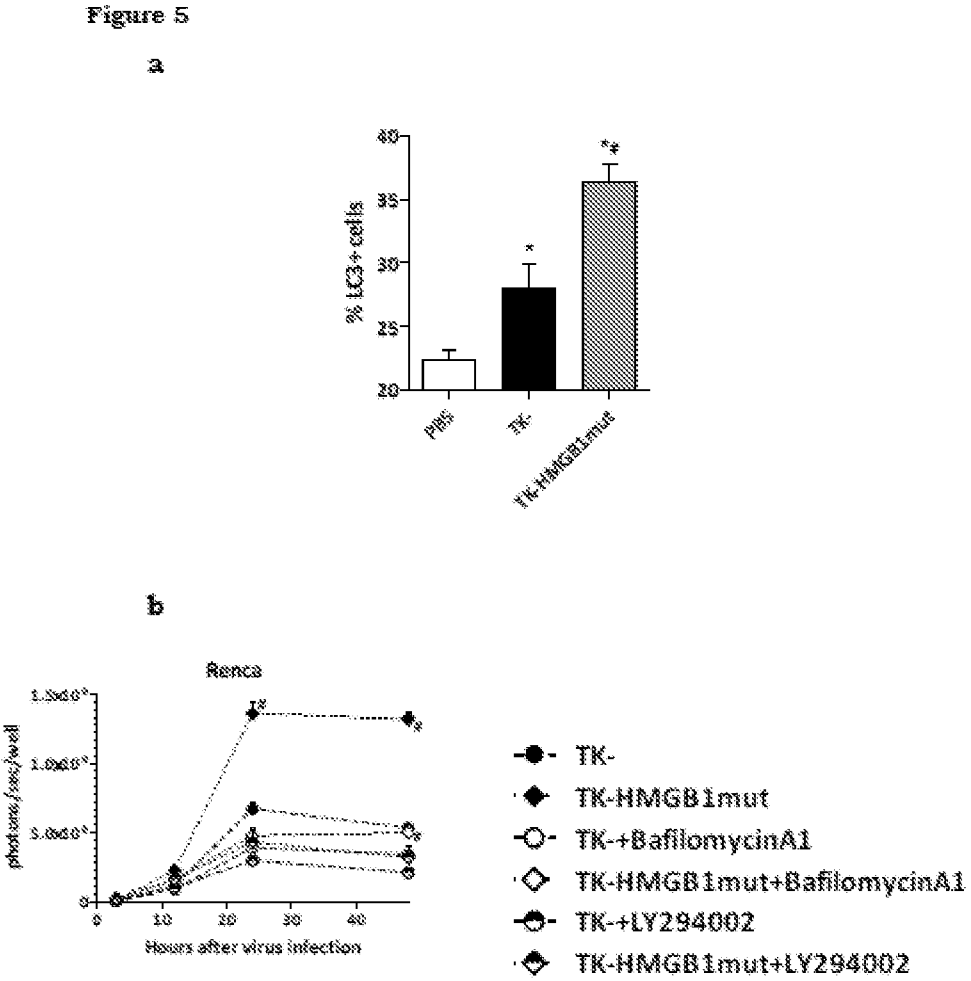


Figure 5
a
% LC3+ cells
PBS
TK-
TK-HMGB1mut
b
Renca
Hours after virus infection
TK-
TK-HMGB1mut
TK-+BafilomycinA1
TK-HMGB1mut+BafilomycinA1
TK-+LY294002
TK-HMGB1mut+LY294002

Cont'd. FIGURE 5

Figure 5b – cont'd.

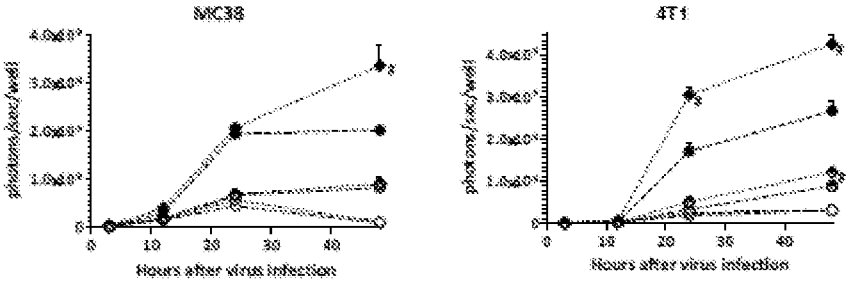
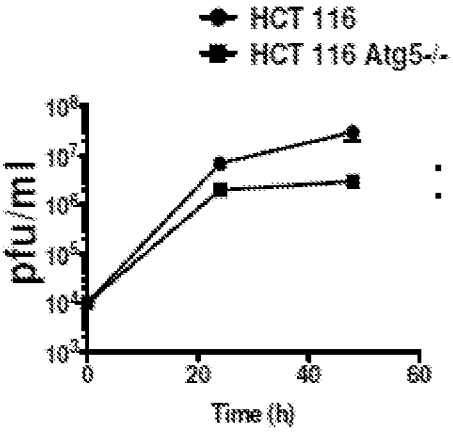


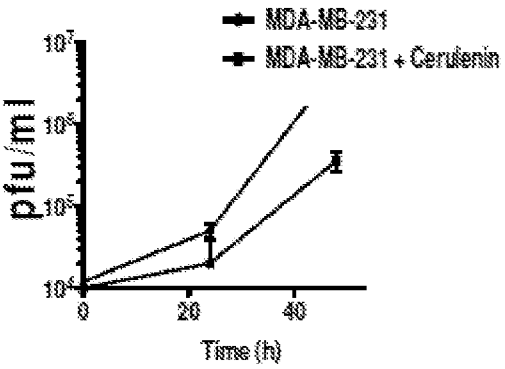
FIGURE 6

Figure 6

a



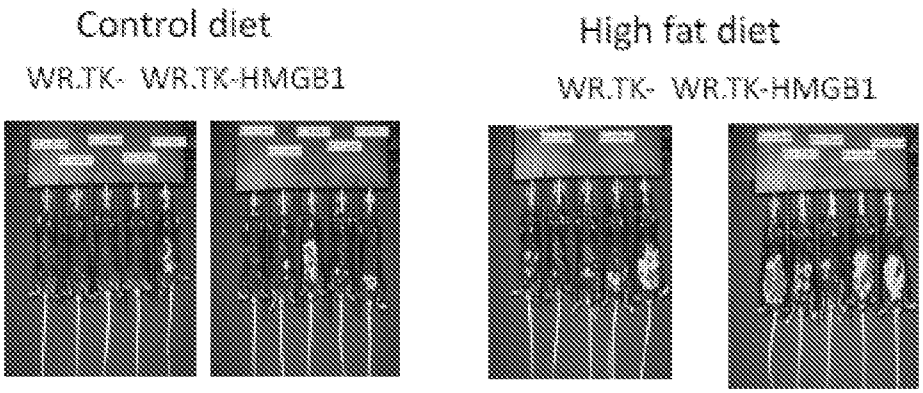
b



Cont'd FIGURE 6

Figure 6 – cont'd.

c



d

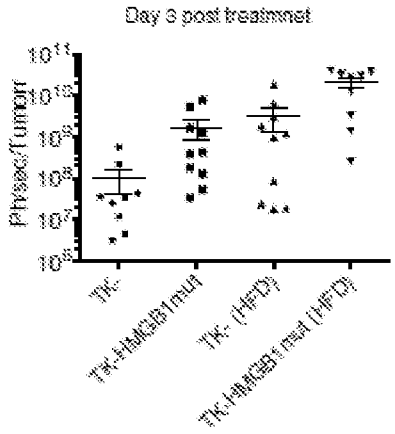


FIGURE 7

Figure 7

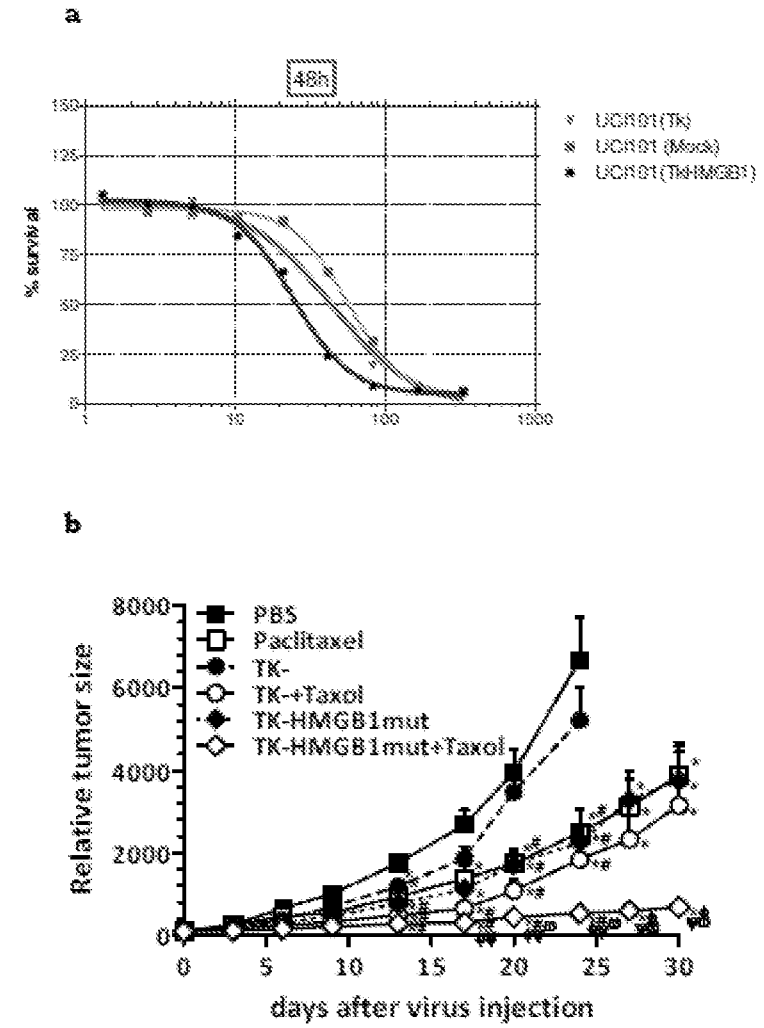


FIGURE 8

Figure 8

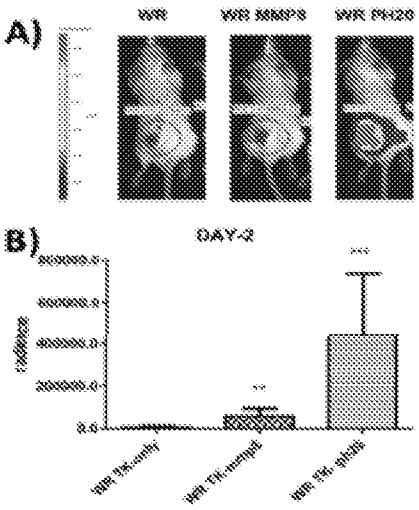


Fig 8 (A) Viral gene expression (BLI) from strains also expressing MMP8 or PH20 and **(B)** data quantified for n=5. PH20 expressing results in increased viral spread

FIGURE 9

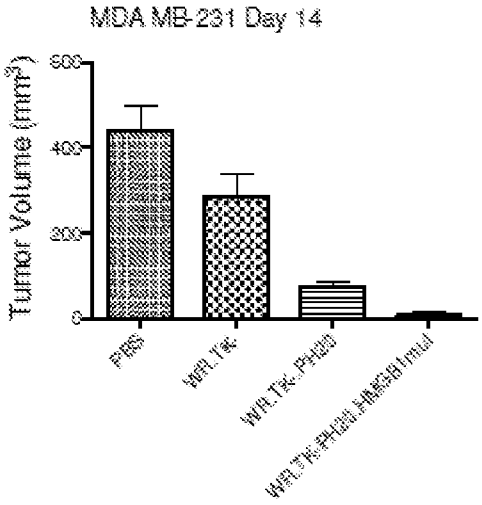

MDA MB-231 Day 14
Tumor Volume (mm³)
800
400
200
0
PBS
WR1Tx
WR1Tx PH20
WR1Tx PH20 HASBH Insul

FIGURE 10

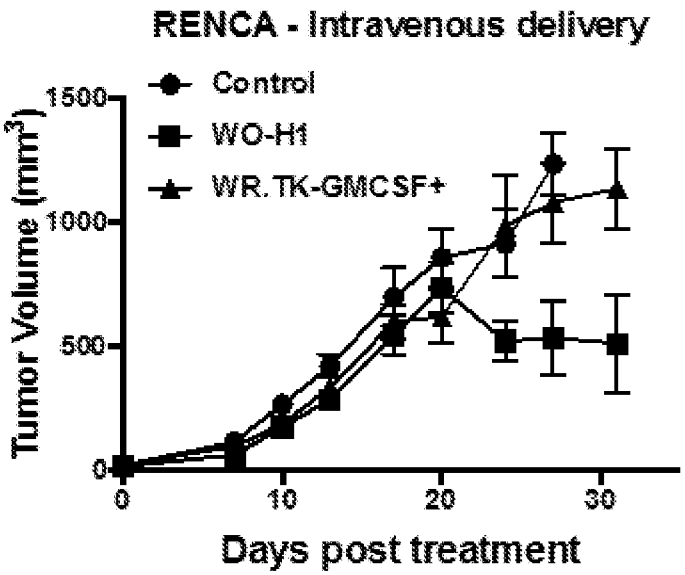

RENCA - Intravenous delivery
Control
WO-H1
WR.TK-GMCSF+
Tumor Volume (mm3)
1500
1000
500
0
0
10
20
30
Days post treatment

FIGURE 11

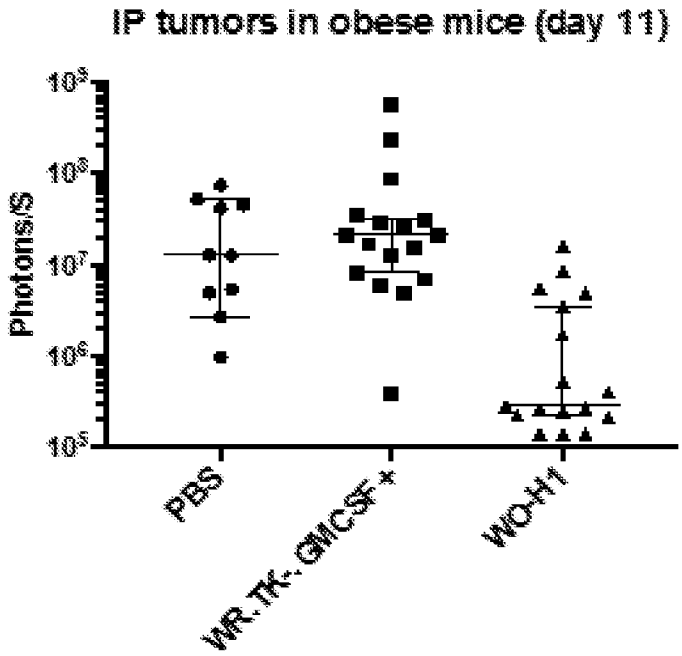
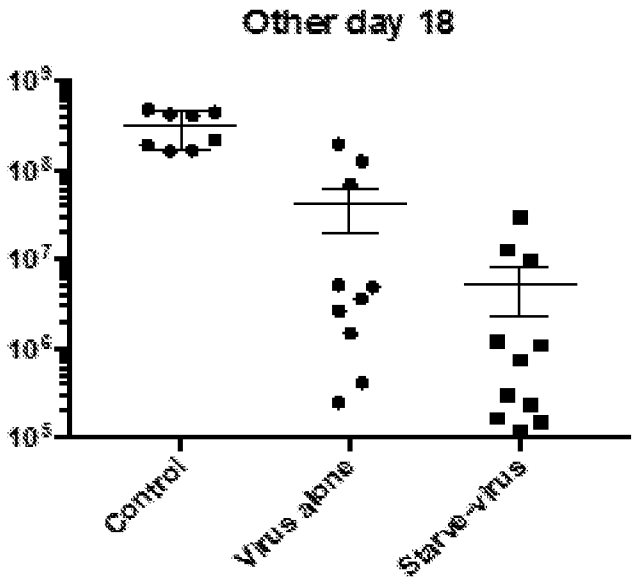

IP tumors in obese mice (day 11)
Photons/S
10^9
10^8
10^7
10^6
10^5
PBS
WR-TK-.GMCSF+
WO-H1

FIGURE 12


Other day 18
10^9
10^8
10^7
10^6
10^5
Control
Virus alone
Starve-virus

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/52746

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/52746

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-6, 11-19, 25-27, 34-37, 41-67, 73-143, 151
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-3, 7-10, 20-24, 28-34, 38-40, 68-72 and 144-150, directed to an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1 or an isolated polynucleotide that codes for a variant HMGB1. The virus and polynucleotide will be searched to the extent that the variant HMGB1 encompasses nucleotide sequence SEQ ID NO: 13 and corresponding amino acid sequence SEQ ID NO: 15. It is believed that claims 1-3, 7-10, 68-72, 144, 145 and 150(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that the variant HMGB1 encompasses SEQ ID NO: 13 and 15. Additional HMGB1 variants will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected HMGB1 variants. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a HMGB1 variant comprising SEQ ID NO: 16 (Claims 20-24, 28-31, 146, 147, 150(in part)). --continued on first extra sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 7-10, 68-72, 144, 145 and 150(in part) limited to SEQ ID NOs: 13, 15

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/52746

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/00, A61K 31/496, A61K 31/5025 (2018.01)
CPC - A61K 31/496, A61K 31/5025, A61K 31/7088, A61K 48/00, A61K 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0183348 A1 (TANIGUCHI et al.) 18 July 2013 (18.07.2013) para [0156]; SEQ ID NO: 4.	68 --- 1-3, 69-72, 144, 150/144
X --- Y	US 2006/0111287 A1 (BIANCHI) 25 May 2006 (25.05.2006) para [0049]; [0086]; [0112]-[0114]; [0148]; [0211]-[0213]; [0276]; [0296]; [0327]; claim 56.	145, 150/145 --- 1-3, 7-10, 70-72, 144, 150/144
Y	US 2016/0235793 A1 (UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 18 August 2016 (18.08.2016) abstract.	1-3, 7-10
Y	US 2005/0152903 A1 (NEWMAN et al.) 14 July 2005 (14.07.2005) para [0023]; SEQ ID NO: 5.	3, 9, 10, 69, (70-72)/69

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 February 2018	13 FEB 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/52746

--continuation of Box III: Observations where unity of invention is lacking--

The inventions listed as Group I+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

No technical features are shared between the HMGB1 variant amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Group I+ were considered to share the technical features of including: an oncolytic vaccinia virus comprising an exogenous nucleic acid that codes for a variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence, wherein the variant HMGB1 comprises a mutation in its first (NLS1), the second nuclear localization signal/sequence (NLS2), or any combinations thereof, and an isolated polynucleotide that codes for a variant HMGB1, these shared technical features are previously disclosed by US 2006/0111287 A1 (Bianchi) in view of US 2016/0235793 A1 to UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION (hereinafter Univ Pittsburgh).

Bianchi teaches a virus comprising an exogenous nucleic acid that codes for a variant HMGB1 (para [0049] "there is provided a method for the prevention of treatment of cancer...comprising administering the protein HMGB1 or a variant or fragment thereof, or a polynucleotide encoding therefor"; [0148] "Delivery of a gene coding for a protein that mimics functional acetylated HMGB1 to appropriate cells is effected in vivo or ex vivo by use of viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus"), wherein the exogenous nucleic acid comprises a nucleotide sequence, wherein the variant HMGB1 comprises a mutation in its first (NLS1), the second nuclear localization signal/sequence (NLS2), or any combinations thereof (para [0011]-[0016] "an isolated acetylated protein HMGB1 derivable from a myeloid cell; or a variant or fragment thereof, or a polynucleotide encoding therefor...at least one nuclear localization signal is acetylated...Preferably the HMGB1 is acetylated on its two nuclear localization signals...an expression vector comprising the polynucleotide of the present invention"; [0086] "The mutation of the lysine clusters within the NLSs to glutamines, which most resemble acetylated lysines, is sufficient to abrogate NLS function"; [0296] "Plasmid pEGFP-HMGB1 was used as template to generate the mutants in NLS1 and NLS2 in two-step PCR mutagenesis"; [0327] "Double mutants of HMGB1-GFP, where both lysine clusters in NLS 1 and NLS2 are changed to glutamines, alanines or arginines, were then constructed. The double NLS1/NLS2 mutant where lysines are changed to arginines"), and an isolated polynucleotide that codes for a variant HMGB1 (claim 56 "An isolated multiply acetylated protein HMGB1, or a variant or fragment thereof, or a polynucleotide encoding therefore"). Bianchi does not specifically teach the virus is an oncolytic vaccinia virus. However, Bianci does teach use of various types of vectors for expressing the variant HMGB1 to illicit an immune response to treat cancer (para [0049] "there is provided a method for the prevention of treatment of cancer...comprising administering the protein HMGB1 or a variant or fragment thereof, or a polynucleotide encoding therefor"; [0148] "Delivery of a gene coding for a protein that mimics functional acetylated HMGB1 to appropriate cells is effected in vivo or ex vivo by use of viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus"; [0211] "method for inducing an immunological response...inoculating the individual with the HMGB1 protein of the present invention"), and administration of the variant causes enhanced cytotoxic T lymphocyte induction (para [0211]-[0213] "a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with the HMGB1 protein of the present invention, or a fragment or variant thereof, adequate to produce antibody and/or T cell immune response to protect said individual from for example a tumor or infection such as a bacterial or viral infection. Also provided are methods whereby such immunological response slows tumor growth...an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells. The immunological response may be to a HMGB1 protein of the present invention").

Univ Pittsburgh teaches use of oncolytic vaccinia viruses which have been modified to promote anti-tumor immunity and/or reduce host immunity and/or antibody response against the virus, wherein the modification is a transgene that enhances cytotoxic T lymphocyte induction (abstract "The present invention relates to oncolytic vaccinia viruses which have been modified to promote anti-tumor immunity and/or reduce host immunity and/or antibody response against the virus. It is based, at least in part, on the discovery that oncolytic vaccinia virus...(iii) carrying a gene that enhances cytotoxic T lymphocyte induction (e.g., TRIF)...reduces tumor growth. Accordingly, the present invention provides for immunooncolytic vaccinia viruses and methods of using them in the treatment of cancers"). Since Bianchi teaches use of the HMGB1 variant to treat cancer by enhancing T lymphocyte induction, it would have been obvious to one of ordinary skill in the art to have used an oncolytic vaccinia virus as taught by Univ Pittsburgh to express the HMGB1 variant to further enhance oncolytic activity of the vector.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, claims 4-6, 11-19, 25-27, 35-37, 41-67, 73-143, 151 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

NOTE, claim 34 which depends from claim 31 or 32, as drafted, is objected to. This is because claim 31 discloses amino acid SEQ ID NO: 16, but does not disclose the antecedent "the exogenous nucleic acid that codes for the variant HMGB1", required in claim 34. Claim 34 is reconstrued to depend from claim 32 or 33.

NOTE, claim 39 which discloses "the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 14", is objected to, because the antecedent claim 38 dicloses SEQ ID NO: 19. Claim 39 is reconstrued to "the variant HMGB1, wherein the exogenous nucleic acid comprises a nucleotide sequence that is at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% homologous to SEQ ID NO: 19".