rapidly cleared and yet are therapeutically effective, pharmaceutical compositions comprising such oncolytic vaccinia viruses, and methods of use of such pharmaceutical compositions, alone or in combination with another agent or therapy, to treat a subject having a cancer.

SUMMARY

The present disclosure is based, at least in part, on the development of pharmaceutical compositions comprising an investigational oncolytic vaccinia virus and the discovery that such compositions are cytotoxic against various types of human cancer cell lines *in vitro*. The present disclosure is also based, at least in part, on the discovery that such pharmaceutical compostions have antitumor activity *in vivo*, that administration of the pharmaceutical compositions to a subject using a specific dosing rgimen is very efficacious (*e.g.,* the discovery that administration on days 1 and 15 is more efficacious as compared to a single administration), that administration of the pharmaceutical compositions to a subject induces intratumoral secretion of murine IL-12, human IL-7 and murine interferon gamma (IFN-γ) proteins and increased tumor infiltration with CD8+ T cells and CD4+ T cells, and that administration of the pharmaceutical compositions of the disclosure in combination with a checkpoint inhibitor, *i.e.,* an anti-PD-1 antibody or an anti-CTLA4 antibody, induced higher antitumor activity than any of the treatments alone. The present disclosure is further based, at least in part, on the discovery that mice that achieved complete tumor regression (CR) following administration of the pharmaceutical compositions of the disclosure rejected the same cancer cells when re-challenged about 90 days after the CR, demonstrating establishment of antitumor immune memory. In addition, the present disclosure is based, at least in part, on the discovery that administration of the pharmaceutical compositions of the disclosure had an abscopal effect in a bilateral tumor model.

Accordingly, in one aspect, the present disclosure provides a pharmaceutical composition comprising, 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier. In one example, the present disclosure provides a pharmaceutical composition comprising about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; and a pharmaceutically acceptable carrier.

In one example, the pharmaceutically acceptable carrier comprises tromethamine and sucrose.

In one example, the pharmaceutically acceptable carrier comprises tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L.

In one example, the pharmaceutically acceptable carrier comprises sucrose at a concentration of about 5% w/v to about 15% w/v.

In one example, the pH of the composition is about 5.0 to about 8.5.

In another aspect, the present disclosure provides a pharmaceutical composition comprising, 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and sucrose at a concentration of 5% w/v to 15% w/v, wherein the pH of the composition is 5.0 to 8.5. In another example, the present disclosure provides a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and sucrose at a concentration of about 5% w/v to about 15% w/v, wherein the pH of the composition is about 5.0 to about 8.5.

In one example, the deletion in the SCR domains in the B5R membrane protein extracellular region comprises a deletion in SCR domains 1-4.

In one example, the deletion in the SCR domains of the B5R region comprises amino acid residues 22-237 of the amino acid sequence set forth in GenBank Accession No. AAA48316.1.

In one example, the gene encoding the SCR domain-deleted B5R region is a gene encoding a polypeptide containing the signal peptide, stalk, transmembrane, and cytoplasmic tail domains of the B5R region.

In one example, the SCR domain-deleted B5R region comprises the amino acid sequence of the B5R region corresponding to the amino acid sequence set forth in SEQ ID NO: 2.

In one example, the vaccinia virus is a LC16mo strain of virus.

In one example, the oncolytic vaccinia virus is LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7.

The pharmaceutical composition of the disclosure may comprise about 1 x 10^7 to about 1 x 10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus; about 1 x 10^7 particle forming units (pfu)/ml of the oncolytic vaccinia virus; about 5 x 10^7 particle forming units (pfu)/ml of the oncolytic vaccinia virus; about 1 x 10^8 particle forming units (pfu)/ml of the oncolytic vaccinia virus; about 5 x 10^8 particle forming units (pfu)/ml of the oncolytic vaccinia virus; about 1 x 10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus; or about 5 x 10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

The pharmaceutical composition of the disclosure may comprise tromethamine at a concentration of about 15 mmol/L to about 45 mmol/L; 20 mmol/L to about 40 mmol/L; or 25 mmol/L to about 35 mmol/L. In one example, the concentration of tromethamine is about 30 mmol/L.

The pharmaceutical composition of the disclosure may comprise sucrose at a concentration of about 6% w/v to about 14% w/v; about 7% w/v to about 13% w/v; about 8% w/v to about 12% w/v; or about 9% w/v to about 11% w/v. In one example, the concentration of sucrose is about 10% w/v.

The pH of the pharmaceutical composition may be about 8.0; about 6.5 to about 8.0; or about 6.8 to about 7.8. In one example, the pH of the composition is about 7.6.

In one example, the composition is stable for at least about 6 months to about 2 years when stored at about -70ºC.

The present disclosure also provides a vial and a syringe comprising any of the pharmaceutical compositions of the disclosure.

In one aspect, the present disclosure provides a vial comprising the pharmaceutical composition as disclosed herein.

In one aspect, the present disclosure provides a syringe comprising the pharmaceutical composition as disclosed herein.

In one aspect, the present disclosure provides the use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier.

In one aspect, the present disclosure provides the use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier, wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

In one aspect, the present disclosure provides the use of an oncolytic vaccinia virus in the manufacture of a medicament for inducing an abscopal effect in a subject having a cancer, wherein the medicament comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and a pharmaceutically acceptable carrier.

In one aspect, the present disclosure provides the use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and sucrose at a concentration of 5% w/v to 15% w/v, wherein the pH of the composition is 5.0 to 8.5.

In one aspect, the present disclosure provides the use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and sucrose at a concentration of 5% w/v to 15% w/v, wherein the pH of the composition is 5.0 to 8.5, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

In one aspect, the present disclosure provides the use of an oncolytic vaccinia virus in the manufacture of a medicament for inducing an abscopal effect in a subject having a cancer, wherein the medicament comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and sucrose at a concentration of 5% w/v to 15% w/v, wherein the pH of the composition is 5.0 to 8.5, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

In one aspect, the present disclosure provides a pharmaceutical composition comprising, 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6.

In one aspect, the present disclosure provides the use of a pharmaceutical composition in the manufacture of a medicament for treating a subject having a cancer, wherein the pharmaceutical composition comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6.

In one aspect, the present disclosure provides the use of a pharmaceutical composition in the manufacture of a medicament for treating a subject having a cancer, wherein the pharmaceutical composition comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

In one aspect, the present disclosure provides the use of a pharmaceutical composition in the manufacture of a medicament for inducing an abscopal effect in a subject having a cancer, wherein the pharmaceutical composition comprises 1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

In one example, the present disclosure provides a method of treating a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; and a pharmaceutically acceptable carrier, thereby treating the subject.

In another example, the present disclosure provides a method of treating a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; and a pharmaceutically acceptable carrier, wherein administration of the pharmaceutical composition to the subject induces an abscopal effect, thereby treating the subject.

In another example, the present disclosure provides a method of inducing an abscopal effect in a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-

IL12/O1L-SP-IL7; and a pharmaceutically acceptable carrier, thereby inducing an abscopal effect in a subject having a cancer.

In one example, the present disclosure method of treating a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition, comprising about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and sucrose at a concentration of about 5% w/v to about 15% w/v, wherein the pH of the composition is about 5.0 to about 8.5, thereby treating the subject.

In another example, the present disclosure provides a method of treating a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and sucrose at a concentration of about 5% w/v to about 15% w/v, wherein the pH of the composition is about 5.0 to about 8.5, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect, thereby treating the subject.

In another example, the present disclosure provides a method of inducing an abscopal effect in a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region, *e.g.,* LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 10 mmol/L to about 50 mmol/L; and sucrose at a concentration of about 5% w/v to about 15% w/v, wherein the pH of the composition is about 5.0 to about 8.5, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect, thereby inducing an abscopal effect in the subject.

In one example, the abscopal effect occurs in a metastatic tumor that is proximate to a primary solid tumor.

In another example, the abscopal effect occurs in a metastatic tumor that is remote to a primary solid tumor.

In one example, the oncolytic vaccinia virus is LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7.

The subject may be administered a dose of about 1 x 10^7 to about 1 x 10^9 particle forming units (pfu); a dose of about 1 x 10^7 particle forming units (pfu); a dose of about 5 x 10^7 particle forming units (pfu); a dose of about 1 x 10^8 particle forming units (pfu); a dose of about 5 x 10^8 particle forming units (pfu); or a dose of about 1 x 10^9 particle forming units (pfu).

In one example, the administration is intratumoral administration.

In one example, the dose of the pharmaceutical composition is administered to the subject intratumorally in a volume that achieves an injection ratio of about 0.2 to about 0.8 (volume of pharmaceutical composition/ tumor volume).

The pharmaceutical composition may be administered to the subject once about once every week, once every two weeks, once every three weeks, or once every four weeks. In one example, the pharmaceutical composition is administered to the subject once about once every two weeks.

The pharmaceutical composition may be administered to the subject in a dosing regimen.

In one example, the dosing regimen comprises administering to the subject a first dose of the pharmaceutical composition on day 1 and a second dose of the pharmaceutical composition on day 15.

In one example, the dosing regimen is repeated beginning at day 28 following the first dose of the pharmaceutical composition.

In one example, the cancer is a primary tumor, such as a solid tumor. In one example, the solid tumor is an advanced solid tumor.

In one example, the cancer is a metastatic tumor.

In one example, the cancer is a cutaneous, subcutaneous, mucosal or submucosal tumor.

In one example, the cancer is a primary or metastatic solid tumor in a location other than a cutaneous, a subcutaneous, a mucosal or a submucosal location.

In one example, the cancer is a head and neck squamous cell carcinoma, a dermatological cancer, a nasopharyngeal cancer, a sarcoma, or a genitourinary/gynecological tumor.

In one example, the cancer is a primary or metastatic tumor of the liver.

In one example, the cancer is a primary or metastatic gastric tumor.

In one example, the cancer the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

In one example, the subject is human.

The human subject may be an adult subject; an adolescent subject; or a pediatric subject.

In one example, administration of the pharmaceutical composition to the subject leads to at least one effect selected from the group consisting of inhibition of tumor growth, tumor regression, reduction in the size of a tumor, reduction in tumor cell number, delay in tumor growth, abscopal effect, inhibition of tumor metastasis, reduction in metastatic lesions over time, reduced use of chemotherapeutic or cytotoxic agents, reduction in tumor burden, increase in progression-free survival, increase in overall survival, complete response, partial response, antitumor immunity, and stable disease.

The methods of the disclosure may further comprise administering to the subject an additional therapeutic agent or therapy,

In one example, the additional therapeutic agent or therapy, is selected from the group consisting of surgery, radiation, a chemotherapeutic agent, a cancer vaccine, a checkpoint inhibitor, a lymphocyte activation gene 3 (LAG3) inhibitor, a glucocorticoid-induced tumor necrosis factor receptor (GITR) inhibitor, a T-cell immunoglobulin and mucin-domain containing-3 (TIM3) inhibitor, a B- and T-lymphocyte attenuator (BTLA) inhibitor, a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor, a CD47 inhibitor, an indoleamine-2,3-dioxygenase (IDO) inhibitor, a bispecific anti-CD3/anti-CD20 antibody, a vascular endothelial growth factor (VEGF) antagonist, an angiopoietin-2 (Ang2) inhibitor, a transforming growth factor beta (TGFβ) inhibitor, a CD38 inhibitor, an epidermal growth factor receptor (EGFR) inhibitor, granulocyte-macrophage colony stimulating factor (GM-CSF), cyclophosphamide, an antibody to a tumor-specific antigen, Bacillus Calmette-Guerin vaccine, a cytotoxin, an interleukin 6 receptor (IL-6R) inhibitor, an interleukin 4 receptor (IL-4R) inhibitor, an IL-10 inhibitor, IL-2, IL-7, IL-21, IL-15, an antibody-drug conjugate, an anti-inflammatory drug, and a dietary supplement.

The methods of the disclosure may further comprise administering to the subject a therapeutically effective amount of a checkpoint inhibitor.

In one example, the checkpoint inhibitor is a programmed cell death 1 (PD-1) inhibitor; a programmed cell death ligand 1 (PD-L1) inhibitor; a cytotoxic T lymphocyte associated protein 4 (CTLA-4) inhibitor; a T-cell immunoglobulin domain and mucin domain-3 (TIM-3) inhibitor; a lymphocyte activation gene 3 (LAG-3) inhibitor; a T cell

immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor; a B and T lymphocyte associated (BTLA) inhibitor; or a V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) inhibitor.

In one example, the checkpoint inhibitor is a programmed cell death 1 (PD-1) inhibitor, a programmed cell death ligand 1 (PD-L1) inhibitor, or a cytotoxic T lymphocyte associated protein 4 (CTLA-4) inhibitor.

In one example, the checkpoint inhibitor is selected from the group consisting of an anti-PD-1 antibody, or antigen-binding fragment thereof; an anti-PD-L1 antibody, or antigen-binding fragment thereof; an anti-CTLA-4 antibody, or antigen-binding fragment thereof; an anti-TIM-3 antibody, or antigen-binding fragment thereof; an anti-LAG-3 antibody, or antigen-binding fragment thereof; an anti-TIGIT antibody, or antigen-binding fragment thereof; an anti-BTLA antibody, or antigen-binding fragment thereof; and an anti-VISTA antibody, or antigen-binding fragment thereof.

In one example, the checkpoint inhibitor is an anti-programmed cell death 1 (PD-1) antibody, or antigen-binding fragment thereof; an anti-programmed cell death ligand 1 (PD-L1) antibody, or antigen-binding fragment thereof; or an anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4) antibody, or antigen-binding fragment thereof.

In one example, the anti-PD-1 antibody is nivolumab or pembrolizumab.

In one example, the anti-PD-L1 antibody is atezolizumab.

In one example, the anti-CTLA-4 antibody is ipilimumab.

In one example, the present disclosure provides a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6.

In one example, the present disclosure provides a method of treating a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6, thereby treating the subject.

In another example, the present disclosure provides a method of treating a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect, thereby treating the subject.

In one example, the present disclosure provides a method of inducing an abscopal effect in a subject having a cancer. The method includes administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising, about 1 x 10^6 to about 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7; tromethamine at a concentration of about 30 mmol/L; and sucrose at a concentration of about 10% w/v, wherein the pH of the composition is about 7.6, and wherein administration of the pharmaceutical composition to the subject induces an abscopal effect, thereby inducing an abscopal effect in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a series of graphs depicting the cytotoxic effect of the hIL12 and hIL7-carrying vaccinia virus against human cancer cell lines. The human cell lines used were: Human cancer cell lines: NCI-H28 (mesothelioma), U-87 MG (glioblastoma), HCT 116 (colorectal carcinoma), A549 (lung carcinoma), DMS 53 (small cell lung cancer cell), GOTO (neuroblastoma), Kato III (gastric cancer cell), OVMANA (ovarian cancer cell), Detroit 562 (head and neck cancer cell), SiHa (cervical cancer cell), BxPC-3 (pancreatic cancer cell), MDA-MB-231 (breast cancer cell), Caki-1 (kidney cancer cell), OE33 (esophageal cancer cell), RPMI 8226 (myeloma), JHH-4 (hepatocellular carcinoma), LNCaP clone FGC (prostate cancer cell), RPMI-7951 (malignant melanoma), JIMT-1 (breast cancer cell), HCC4006 (lung adenocarcinoma), SK-OV-3 (ovarian cancer cell), RKO (colon cancer cell), 647-V (bladder cancer cell) and NCI-H226 (lung squamous cell carcinoma).

Figure 2 is a graph depicting the replication of the hIL12 and hIL7-carrying vaccinia virus genome in human cancer cells or normal cells. Values were normalized to the 18s ribosomal RNA gene and expressed as the mean of duplicate measures. NCI-H520, HARA, LK-2 and LUDLU-1 are human cancer cell lines.

Figure 3A is a graph depicting tumor growth change (tumor volume) in COLO 741 Tumor cell-bearing mice treated with the hIL12 and hIL7-carrying vaccinia virus . Each point represents the mean ± SEM (n = 6). Statistical analysis was performed for the values on day 21. COLO 741: human colorectal carcinoma cell line; Vehicle: 30 mmol/L Tris-HCl containing 10% sucrose. ** $P < 0.01$ compared with the vehicle treatment group (Dunnett's multiple comparison test).

Figure 3B is a graph depicting body weight change in COLO 741 Tumor cell-bearing mice treated with the hIL12 and hIL7-carrying vaccinia virus. Each point represents the mean ± SEM (n = 6). ** $P < 0.01$ compared with the vehicle treatment group (Dunnett's multiple comparison test).

Figure 4A is a graph depicting tumor growth (tumore volume) a change in U-87 MG-bearing mice treated with the hIL12 and hIL7-carrying vaccinia virus. Each point represents

We claim:

1. A pharmaceutical composition comprising,

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and

a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein the pharmaceutically acceptable carrier comprises tromethamine and sucrose.

3. The pharmaceutical composition of claim 2, wherein the pharmaceutically acceptable carrier comprises tromethamine at a concentration of 10 mmol/L to 50 mmol/L.

4. The pharmaceutical composition of claim 2 or 3, wherein the pharmaceutically acceptable carrier comprises sucrose at a concentration of 5% w/v to 15% w/v.

5. The pharmaceutical composition of any one of claims 1-4, wherein the pH of the composition is 5.0 to 8.5.

6. A pharmaceutical composition comprising,

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of an oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region;

tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and

sucrose at a concentration of 5% w/v to 15% w/v,

wherein the pH of the composition is 5.0 to 8.5.

7. The pharmaceutical composition of any one of claims 1-6, wherein the deletion in the SCR domains in the B5R membrane protein extracellular region comprises a deletion in SCR domains 1-4.

8. The pharmaceutical composition of any one of claims 1-7, wherein the deletion in the SCR domains of the B5R region comprises amino acid residues 22-237 of the amino acid sequence set forth in GenBank Accession No. AAA48316.1.

9. The pharmaceutical composition of any one of claims 1-8, wherein the gene encoding the SCR domain-deleted B5R region is a gene encoding a polypeptide containing the signal peptide, stalk, transmembrane, and cytoplasmic tail domains of the B5R region.

10. The pharmaceutical composition according to any one of claims 1-9, wherein the SCR domain-deleted B5R region comprises the amino acid sequence of the B5R region corresponding to the amino acid sequence set forth in SEQ ID NO: 2.

11. The pharmaceutical composition of any one of claims 1-10, wherein the vaccinia virus is a LC16mo strain of virus.

12. The pharmaceutical composition of any one of claims 1-11, wherein the oncolytic vaccinia virus is LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7.

13. The pharmaceutical composition of any one of claims 1-12, comprising 1 x 10^7 to 1 x 10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

14. The pharmaceutical composition of any one of claims 1-12, comprising about 1 x 10^7 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

15. The pharmaceutical composition of any one of claims 1-12, comprising about 5 x 10^7 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

16. The pharmaceutical composition of any one of claims 1-12, comprising about 1 x 10^8 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

17. The pharmaceutical composition of any one of claims 1-12, comprising about 5 x 10^8 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

18. The pharmaceutical composition of any one of claims 1-12, comprising about 1 x 10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

19. The pharmaceutical composition of any one of claims 1-12, comprising about 5 x 10^9 particle forming units (pfu)/ml of the oncolytic vaccinia virus.

20. The pharmaceutical composition of any one of claims 2-19, wherein the concentration of tromethamine is 15 mmol/L to 45 mmol/L; 20 mmol/L to about 40 mmol/L; or 25 mmol/L to about 35 mmol/L.

21. The pharmaceutical composition of claim 20, wherein the concentration of tromethamine is about 30 mmol/L.

22. The pharmaceutical composition of any one of claims 4-21, wherein the concentration of sucrose is 6% w/v to 14% w/v; 7% w/v to 13% w/v; 8% w/v to 12% w/v; or 9% w/v to 11% w/v.

23. The pharmaceutical composition of claim 22, wherein the concentration of sucrose is about 10% w/v.

24. The pharmaceutical composition of any of claims 5-23, wherein the pH of the composition is 6.0 to 8.0; 6.5 to 8.0; or 6.8 to 7.8.

25. The pharmaceutical composition of claim 24, wherein the pH of the composition is about 7.6.

26. The pharmaceutical composition of any one of claims 1-25, wherein the composition is stable for at least 6 months to 2 years when stored at about -70°C.

27. A vial comprising the pharmaceutical composition of any one of claims 1-26.

28. A syringe comprising the pharmaceutical composition of any one of claims 1-26.

29. Use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and

a pharmaceutically acceptable carrier.

30. Use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and

a pharmaceutically acceptable carrier,

wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

31. Use of an oncolytic vaccinia virus in the manufacture of a medicament for inducing an abscopal effect in a subject having a cancer, wherein the medicament comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region; and

a pharmaceutically acceptable carrier.

32. Use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region;

tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and

sucrose at a concentration of 5% w/v to 15% w/v,

wherein the pH of the composition is 5.0 to 8.5.

33. Use of an oncolytic vaccinia virus in the manufacture of a medicament for treating a subject having a cancer, wherein the medicament comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region;

tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and

sucrose at a concentration of 5% w/v to 15% w/v,

wherein the pH of the composition is 5.0 to 8.5, and

wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

34. Use of an oncolytic vaccinia virus in the manufacture of a medicament for inducing an abscopal effect in a subject having a cancer, wherein the medicament comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of the oncolytic vaccinia virus, wherein the oncolytic vaccinia virus comprises in its genome a polynucleotide encoding human interleukin-7 and a polynucleotide encoding human interleukin-12, lacks a functional virus growth factor (VGF) protein and a functional O1L protein, and has a deletion in the SCR domains in the B5R membrane protein extracellular region;

tromethamine at a concentration of 10 mmol/L to 50 mmol/L; and

sucrose at a concentration of 5% w/v to 15% w/v,

wherein the pH of the composition is 5.0 to 8.5, and

wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

35. The use of any one of claims 30, 31, 33, and 34, wherein the abscopal effect occurs in a metastatic tumor that is proximate to a primary solid tumor.

36. The use of any one of claims 30, 31, 33, and 34, wherein the abscopal effect occurs in a metastatic tumor that is remote to a primary solid tumor.

37. The use of any one of claims 29-36, wherein the oncolytic vaccinia virus is LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7.

38. The use of any one of claims 29-37, wherein the subject is to be administered a dose of 1×10^7 to 1×10^9 particle forming units (pfu).

39. The use of any one of claims 29-37, wherein the subject is to be administered a dose of about 1×10^7 particle forming units (pfu).

40. The use of any one of claims 29-37, wherein the subject is to be administered a dose of about 5×10^7 particle forming units (pfu).

41. The use of any one of claims 29-37, wherein the subject is to be administered a dose of about 1 x 10^8 particle forming units (pfu).

42. The use of any one of claims 29-37, wherein the subject is to be administered a dose of about 5 x 10^8 particle forming units (pfu).

43. The use of any one of claims 29-37, wherein the subject is to be administered a dose of about 1 x 10^9 particle forming units (pfu).

44. The use of any one of claims 29-37, wherein the administration is intratumoral administration.

45. The use of any one of claims 29-44, wherein the dose of the pharmaceutical composition is to be administered to the subject intratumorally in a volume that achieves an injection ratio of 0.2 to 0.8 (volume of pharmaceutical composition/ tumor volume).

46. The use of any one of claims 29-44, wherein the pharmaceutical composition is to be administered to the subject once about once every week, once every two weeks, once every three weeks, or once every four weeks.

47. The use of any one of claims 29-44, wherein the pharmaceutical composition is to be administered to the subject once about once every two weeks.

48. The use of any one of claims 29-47, wherein the pharmaceutical composition is to be administered to the subject in a dosing regimen.

49. The use of claim 48, wherein the dosing regimen comprises a first dose of the pharmaceutical composition on day 1 and a second dose of the pharmaceutical composition on day 15.

50. The use of claim 49, wherein the dosing regimen is repeated beginning at day 28 following the first dose of the pharmaceutical composition.

51. The use of any one of claims 29-50, wherein the cancer is a primary tumor.

52. The use of claim 51, wherein the primary tumor is a solid tumor.

53. The use of claim 50, wherein the solid tumor is an advanced solid tumor.

54. The use of any one of claims 29-50, wherein the cancer is a metastatic tumor.

55. The use of any one of claims 29-50, wherein the cancer is a cutaneous, subcutaneous, mucosal or submucosal tumor.

56. The use of any one of claims 29-50, wherein the cancer is a primary or metastatic solid tumor in a location other than a cutaneous, a subcutaneous, a mucosal or a submucosal location.

57. The use of any one of claims 29-50, wherein the cancer is a head and neck squamous cell carcinoma, a dermatological cancer, a nasopharyngeal cancer, a sarcoma, or a genitourinary/gynecological tumor.

58. The use of any one of claims 29-50, wherein the cancer is a primary or metastatic tumor of the liver.

59. The use of any one of claims 29-50, wherein the cancer is a primary or metastatic gastric tumor.

60. The use of any one of claims 29-50, wherein the cancer the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

61. The use of any one of claims 29-60, wherein the subject is human.

62. The use of any one of claims 29-61, wherein the subject is an adult subject.

63. The use of any one of claims 29-61, wherein the subject is an adolescent subject.

64. The use of any one of claims 29-61, wherein the subject is a pediatric subject.

65. The use of any one of claims 29-64, wherein administration of the pharmaceutical composition to the subject leads to at least one effect selected from the group consisting of inhibition of tumor growth, tumor regression, reduction in the size of a tumor,

reduction in tumor cell number, delay in tumor growth, abscopal effect, inhibition of tumor metastasis, reduction in metastatic lesions over time, reduced use of chemotherapeutic or cytotoxic agents, reduction in tumor burden, increase in progression-free survival, increase in overall survival, complete response, partial response, antitumor immunity, and stable disease.

66. The use of any one of claims 29-65, further comprising an additional therapeutic agent or therapy,

67. The use of claim 66, wherein the additional therapeutic agent or therapy, is selected from the group consisting of surgery, radiation, a chemotherapeutic agent, a cancer vaccine, a checkpoint inhibitor, a lymphocyte activation gene 3 (LAG3) inhibitor, a glucocorticoid-induced tumor necrosis factor receptor (GITR) inhibitor, a T-cell immunoglobulin and mucin-domain containing-3 (TIM3) inhibitor, a B- and T-lymphocyte attenuator (BTLA) inhibitor, a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor, a CD47 inhibitor, an indoleamine-2,3-dioxygenase (IDO) inhibitor, a bispecific anti-CD3/anti-CD20 antibody, a vascular endothelial growth factor (VEGF) antagonist, an angiopoietin-2 (Ang2) inhibitor, a transforming growth factor beta (TGFβ) inhibitor, a CD38 inhibitor, an epidermal growth factor receptor (EGFR) inhibitor, granulocyte-macrophage colony stimulating factor (GM-CSF), cyclophosphamide, an antibody to a tumor-specific antigen, Bacillus Calmette-Guerin vaccine, a cytotoxin, an interleukin 6 receptor (IL-6R) inhibitor, an interleukin 4 receptor (IL-4R) inhibitor, an IL-10 inhibitor, IL-2, IL-7, IL-21, IL-15, an antibody-drug conjugate, an anti-inflammatory drug, and a dietary supplement.

68. The use of any one of claims 30-67, further comprising a checkpoint inhibitor.

69. The use of claim 68, wherein the checkpoint inhibitor is a programmed cell death 1 (PD-1) inhibitor; a programmed cell death ligand 1 (PD-L1) inhibitor; a cytotoxic T lymphocyte associated protein 4 (CTLA-4) inhibitor; a T-cell immunoglobulin domain and mucin domain-3 (TIM-3) inhibitor; a lymphocyte activation gene 3 (LAG-3) inhibitor; a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor; a B and T lymphocyte associated (BTLA) inhibitor; or a V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) inhibitor.

70. The use of claim 69, wherein the checkpoint inhibitor is a programmed cell death 1 (PD-1) inhibitor, a programmed cell death ligand 1 (PD-L1) inhibitor, or a cytotoxic T lymphocyte associated protein 4 (CTLA-4) inhibitor.

71. The use of claim 69, wherein the checkpoint inhibitor is selected from the group consisting of an anti-PD-1 antibody, or antigen-binding fragment thereof; an anti-PD-L1 antibody, or antigen-binding fragment thereof; an anti-CTLA-4 antibody, or antigen-binding fragment thereof; an anti-TIM-3 antibody, or antigen-binding fragment thereof; an anti-LAG-3 antibody, or antigen-binding fragment thereof; an anti-TIGIT antibody, or antigen-binding fragment thereof; an anti-BTLA antibody, or antigen-binding fragment thereof; and an anti-VISTA antibody, or antigen-binding fragment thereof.

72. The use of claim 71, wherein the checkpoint inhibitor is an anti-programmed cell death 1 (PD-1) antibody, or antigen-binding fragment thereof; an anti-programmed cell death ligand 1 (PD-L1) antibody, or antigen-binding fragment thereof; or an anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4) antibody, or antigen-binding fragment thereof.

73. The use of claim 72, wherein the anti-PD-1 antibody is nivolumab or pembrolizumab.

74. The use of claim 72, wherein the anti-PD-L1 antibody is atezolizumab.

75. The use of claim 72, wherein the anti-CTLA-4 antibody is ipilimumab.

76. A pharmaceutical composition comprising,

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7;

tromethamine at a concentration of about 30 mmol/L; and

sucrose at a concentration of about 10% w/v,

wherein the pH of the composition is about 7.6.

77. Use of a pharmaceutical composition in the manufacture of a medicament for treating a subject having a cancer, wherein the pharmaceutical composition comprises

1×10^6 to 1×10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7;

tromethamine at a concentration of about 30 mmol/L; and

sucrose at a concentration of about 10% w/v,

wherein the pH of the composition is about 7.6.

78. Use of a pharmaceutical composition in the manufacture of a medicament for treating a subject having a cancer, wherein the pharmaceutical composition comprises

1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7;

tromethamine at a concentration of about 30 mmol/L; and

sucrose at a concentration of about 10% w/v,

wherein the pH of the composition is about 7.6, and

wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.

79. Use of a pharmaceutical composition in the manufacture of a medicament for inducing an abscopal effect in a subject having a cancer, wherein the pharmaceutical composition comprises

1 x 10^6 to 1 x 10^{10} particle forming units (pfu)/ml of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7;

tromethamine at a concentration of about 30 mmol/L; and

sucrose at a concentration of about 10% w/v,

wherein the pH of the composition is about 7.6, and

wherein administration of the pharmaceutical composition to the subject induces an abscopal effect.