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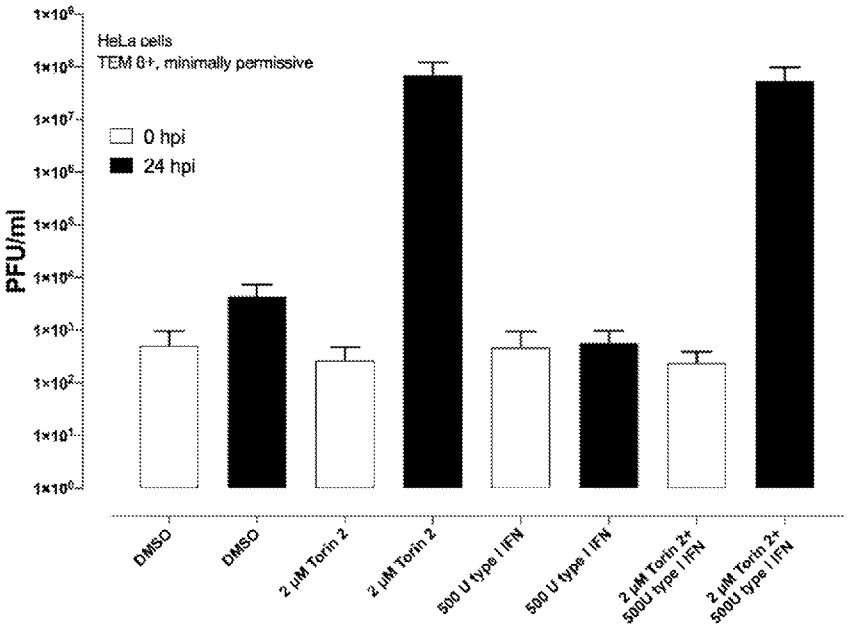


FIG. 4A

(57) Abstract: Provided herein are compositions and methods of using Seneca Valley Virus (SVV) or a derivative thereof and an interferon type I (IFN-I) inhibiting agent comprising an mTOR inhibitor for treating a cancer in a subject. The disclosed methods particularly rely upon the expression level of an ANTXR1 and the expression level of IFN-I in the cancer from the subject. Also provided herein are methods for predicting the efficacy of an SVV treatment comprising IFN-I inhibiting agent.

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SECOND GENERATION SENECA VALLEY VIRUS ONCOLYTIC THERAPY: COMPOSITIONS AND METHODS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application number 62/876,191, filed July 19, 2019, the disclosure of which is incorporated herein by reference in its entirety.

GOVERNMENT RIGHTS

[0002] This invention was made with government support under Contract No. R21AI139775 awarded by the National Institutes of Health (NIH) and National Institute of Allergy and Infectious Diseases (NIAID). The government may have certain rights in the invention.

TECHNICAL FIELD

[0003] The disclosed invention is in the field of oncolytic viruses and their use in treating cancer.

BACKGROUND

[0004] Cancer is the second most common cause of death in the United States. One out of every four individuals dies from it, and more than one million new cancer diagnoses are made every year. The disease begins with the uncontrolled proliferation and growth of abnormal, transformed cells. However, the definition does not end with a description of one disease but of hundreds of different diseases. No two cancers are the same, nor are they clonal. The mutations driving and acquired during cell transformation may be similar, but they are never identical. This conundrum adds to the complexity and heterogeneity of the pathologies that patients develop. Current cancer therapies, including chemotherapeutics and radiation, are most effective when combined with immunomodulatory agents to create and enhance the antitumor microenvironment. Many malignancies may be resistant to treatment via these traditional

methods. Oncolytic viruses show great potential as anti-cancer agents. The picornavirus Seneca Valley virus (SVV) is a single stranded (+) RNA virus that has been investigated as an oncolytic therapy. It has been shown that SVV can target and facilitate regression of many intractable malignancies, including small and non-small cell lung cancers and pediatric solid tumors. However, a significant number of tumors did not regress after virus infection.

[0005] There is a continuing need to improve upon current designs of oncolytic viruses for developing anti-cancer agents, and for treating cancer.

SUMMARY

[0006] Provided herein are methods of treating a cancer in a subject in need thereof. The methods comprise administering to the subject an interferon type I (IFN-I) inhibiting agent comprising an mTOR inhibitor and an effective amount of Seneca Valley Virus (SVV) or SVV derivative, wherein the cancer is characterized by: an expression level of anthrax toxin receptor 1 (ANTXRl) higher than an ANTXRl reference value, and an expression level of IFN-I higher than an IFN-I reference value.

[0007] Cancers treatable by the disclosed inventions include a triple negative breast cancer, small cell lung cancer, a non-small squamous cell carcinoma, a skin cancer, a hepatocellular carcinoma, a colon cancer, a cervical cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a thyroid cancer, a kidney cancer, a bone cancer, an oesophagus cancer, a soft tissue cancer or any cancer expressing ANTXR1.

[0008] Also provided herein are other methods of treating a cancer in a subject in need thereof. The methods comprise administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor and an effective amount of SVV or SVV derivative, wherein the cancer is characterized by an expression level of ANTXRl higher than an ANTXRl reference value, and wherein the IFN-I inhibiting agent reduces the expression level of IFN-I in the cancer thereby favoring replication of the SVV or the SVV derivative and reducing or eliminating the cancer.

[0009] Also provided herein are methods of predicting the efficacy of a Seneca Valley Virus (SVV) treatment, or an SVV derivative treatment of a cancer in a subject in need thereof. The methods comprise determining the expression level of ANTXRl and the expression level of IFN-I in the cancer from the subject, wherein: an expression level of ANTXRl higher than an ANTXRl reference value, and an expression level of IFN-I higher than an IFN-I reference value

are predictive that the treatment is effective, and wherein when the treatment is predicted to be effective, recommending treatment of the subject; wherein the treatment comprises administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor.

[0010] Also provided herein are pharmaceutical compositions for treating a cancer in a subject in need thereof. The pharmaceutical compositions comprise an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier.

[0011] Further provided herein are methods of using a pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative for treating and/or in the manufacture of a drug for treating cancer in a subject in need thereof.

[0012] The general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as defined in the appended claims. Other aspects of the present invention will be apparent to those skilled in the art in view of the detailed description of the invention as provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings' exemplary embodiments of the invention. However, the invention is not limited to the specific methods and compositions disclosed and the invention is not limited to the precise arrangements and instrumentalities of the embodiments depicted in the drawings. In addition, the drawings are not necessarily drawn to scale. In the drawings:

[0014] Figure 1 is a histogram depicting the inhibition of SVV replication by IFN-α. Per.C6 cells were pretreated with 500 units IFN-α before infection with SVV at MOI=1. Virus yields were determined by plaque assay 24 h post infection. The results are representative of 3 independent experiments.

[0015] Figures 2A-2B are series of histogram depicting the effect of staurosporine on SVV replication. Figure 2A. SVV replication in HeLa cells can be enhanced with staurosporine (SSP). Figure 2B. Inhibition of SVV replication by IFN-α can be reversed with SSP in Per.C6

cells. TEM 8+ HeLa cells or Per.C6 cells were pretreated with 500 units IFN-α before infection with SVV at MOI 1. 300 nM SSP was added at time 0. Virus yields determined by plaque assay 24 h post infection. Results are representative of three independent experiments.

[0016] Figures 3A-3B are series of histogram depicting the effect of Trichostatin A on SVV replication. Figure 3A. SVV replication in HeLa cells can be enhanced with Trichostatin A. Figure 3B. Inhibition of SVV replication by IFN-α can be reversed with Trichostatin A in Per.C6 cells. TEM 8+ HeLa cells or Per.C6 cells were pretreated with 500 units IFN-α before infection with SVV at MOI 1. 1 μM Trichostatin A was added at time 0. Virus yields determined by plaque assay 24 h post infection. Results are representative of three independent experiments.

[0017] Figures 4A-4B are series of histogram depicting the effect of Torin 2 A on SVV replication. Figure 4A. SVV replication in HeLa cells can be enhanced with Torin 2 A. Figure 4B. Inhibition of SVV replication by IFN-α can be reversed with Torin 2 in Per.C6 cells. TEM 8+ HeLa cells or Per.C6 cells were pretreated with 500 units IFN-α before infection with SVV at MOI 1. 2 μM Torin 2 was added at time -30 min. Virus yields determined by plaque assay 24 h post infection. Results are representative of three independent experiments.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as defined in the appended claims. Other aspects of the present invention will be apparent to those skilled in the art in view of the detailed description of the invention as provided herein.

[0019] The present invention relates to compositions and methods of using Seneca Valley Virus (SVV) or a derivative thereof and an interferon type I (IFN-I) inhibiting agent comprising an mTOR inhibitor for treating cancer in a subject. The disclosed methods particularly rely upon the level of an ANTXRl expression and the level of IFN-I expression in a cancerous tissue from the subject. Also provided herein are methods for predicting the efficacy of an SVV treatment comprising IFN-I inhibiting agent.

Definitions

[0020] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention

pertains. Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

[0021] It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0022] As used herein, the articles "a" and "an" are used to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0023] As used herein the terms anthrax toxin receptor 1 (ANTXR1) or Tumor Endothelial Marker 8 (TEM8), are used interchangeably. The protein encoded by ANTXR1 gene is a type I transmembrane protein. ANTXR1/TEM8 is a tumor-specific endothelial marker that has been implicated tumor vasculature formation of various cancers (e.g. colorectal).

[0024] As used herein when referring to a measurable value such as an amount, a temporal duration, and the like, the term "about" is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0025] The term "biological" or "biological sample" refers to a sample obtained from an organism or from components (e.g., cells) of an organism. The sample may be of any biological tissue or fluid. Frequently the sample will be a "clinical sample" which is a sample derived from a patient. Such samples include, but are not limited to, bone marrow, cardiac tissue, sputum, blood, lymphatic fluid, blood cells (e.g., white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

[0026] As used herein, the terms "derivative" specifies that a derivative of a virus can have a nucleic acid or amino acid sequence difference in respect to a template viral nucleic acid or amino acid sequence. For instance, an SVV derivative can refer to an SVV that has a nucleic acid or amino acid sequence different with respect to the wild-type SVV nucleic acid or amino acid sequence of ATCC Patent Deposit Number PTA-5343. In some embodiments, the SVV derivative encompasses an SVV mutant, an SVV variant or a modified SVV (e.g. genetically engineered SVV or a SVV with a transgene). In some embodiments, the modify SVV derivative

is modified to be capable of recognizing different cell receptors (e.g. various cancer antigens or neoantigens) or to be capable of evading the immune system while still being able to invade, replicate and kill the cell of interest (e.g. cancer cell). In general, an SVV or SVV derivative can be derived from an already pre-existing stock of virus that is passaged to produce more viruses. SVV or SVV derivative can also be derived from a plasmid.

[0027] As used herein, "higher" refers to expression levels which are at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% higher or more, and/or 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, 2.0 fold higher or more, and any and all whole or partial increments therebetween, than a control reference. A disclosed herein an expression level higher than a reference value refers to an expression level (mRNA or protein) that is higher than a normal or control level from an expression (mRNA or protein) measured in a healthy subject or defined or used in the art.

[0028] As used herein, "lower" refers to expression levels which are at least 10% lower or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% lower or more, and/or 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, 2.0 fold lower or more, and any and all whole or partial increments in between, than a control reference. A disclosed herein an expression level lower than a reference value refers to an expression level (mRNA or protein) that is lower than a normal or control level from an expression (mRNA or protein) measured in a healthy subject or defined or used in the art.

[0029] As used herein, the terms "control," or "reference" can be used interchangeably and refer to a value that is used as a standard of comparison.

[0030] As used herein, by "combination therapy" is meant that a first agent is administered in conjunction with another agent. "In combination with" or "In conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in combination with" refers to administration of one treatment modality before, during, or after delivery of the other treatment modality to the individual. Such combinations are considered to be part of a single treatment regimen or regime.

[0028] As used herein, the terms "comprising," "including," "containing" and "characterized by" are exchangeable, inclusive, open-ended and do not exclude additional, unrecited elements or method steps. Any recitation herein of the term "comprising," particularly in a description of components of a composition or in a description of elements of a device, is

understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements.

[0029] As used herein, the term "consisting of" excludes any element, step, or ingredient not specified in the claim element.

[0031] The term "Type I interferon (IFN-I)" refers herein to interferon proteins or genes involved in regulating the activity of the immune system with antiviral, antitumor and immunoregulatory functions. As used herein, IFN-I includes any set of proteins, genes or transcripts that comprise or regulate the type I IFN pathway. Examples of IFN-I in mammals include but are not limited to IFN-α (alpha), IFN-β (beta), IFN-κ (kappa), IFN-δ (delta), IFN-ε (epsilon), IFN-τ (tau), IFN-ω (omega), and IFN-ζ (zeta). IFN-I biomarkers (mRNA or protein) may include various cytokines (interferons, interleukins or other growth factors). For instance, IFN-I biomarkers can comprise IFI35, IFN-α, IFN-β, IFN-κ, IFN-δ, IFN-ε, IFN-τ, IFN-ω, and IFN-ζ, ADAR, IRF9, IFITM3, IFITM2, USP18, LOC144383, EGR1, IFI6, GBP2, HLA-A, HLA-B, HLA-C, HLA-F, HLA-G, IRF8, IFI27, IFI35, IFIT2, IFIT1, IFIT3, IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNAR1, IFNAR2, IFNB1, IRF1, IRF2, IRF3, IRF4, IRF5, IRF6, IRF7, ISG20, JAK1, MX1, MX2, OAS1, OAS2, OAS3, IP6K2, XAF1, PSMB8, PTPN1, PTPN6, RNASEL, HLA-K, STAT1, STAT2, TYK2, HLA-B, IFITM1, OASL, SOCS1, SOCS3 and ISG15.

As used herein, the term mammalian target of rapamycin (mTOR) refers to a serine/threonine protein kinase known to play a role in regulating cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription. mTOR is sometimes also referred to as the mechanistic target of rapamycin and FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1), which is encoded in humans by the MTOR gene. mTOR functions as a catalytic subunit for two distinct molecular complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is composed of regulatory associated protein of mTOR (Raptor) and mammalian LST8/G-protein β-subunit like protein (mLST8/GβL). This complex functions as a nutrient/energy/redox sensor and plays a role in regulating protein synthesis. The activity of mTORC1 is stimulated by insulin, growth factors, serum, phosphatidic acid, amino acids (particularly leucine) and oxidative stress. In contrast, mTORC1 is known to be inhibited by low nutrient levels, growth factor deprivation, reductive stress, caffeine, rapamycin, farnesylthiosalicylic acid and curcumin. The components of mTORC2 are rapamycin-insensitive

companion of mTOR (Rictor), GβL, mammalian stress-activated protein kinase interacting protein 1 and mTOR. mTORC2 has been shown to function as an important regulator of the cytoskeleton through its stimulation of F-actin stress fibers, paxillin, RhoA, Rac1, Cdc42 and protein kinase C alpha. Unlike mTORC1, mTORC2 is not sensitive to rapamycin.

[0032] As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that may comprise a protein or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0033] As used herein, plaque forming units (PFU) refers to a measure of number of infectious virus particles. It is determined by plaque forming assay.

[0034] As used herein, multiplicity of infection (MOI) refers the average number of virus particles infecting each cell. MOI can be related to PFU by the following formula:

[0035] Multiplicity of infection (MOI) = Plaque forming units (PFU) of virus used for infection / number of cells.

[0036] The term "RNA" as used herein is defined as ribonucleic acid.

[0037] The term "treatment" as used within the context of the present invention is meant to include therapeutic treatment as well as prophylactic, or suppressive measures for the disease or disorder. As used herein, the term "treatment" and associated terms such as "treat" and "treating" means the reduction of the progression, severity and/or duration of a disease condition or at least one symptom thereof. The term 'treatment' therefore refers to any regimen that can benefit a subject. The treatment may be in respect of an existing condition or may be prophylactic (preventative treatment). Treatment may include curative, alleviative or

prophylactic effects. References herein to "therapeutic" and "prophylactic" treatments are to be considered in their broadest context. The term "therapeutic" does not necessarily imply that a subject is treated until total recovery. Similarly, "prophylactic" does not necessarily mean that the subject will not eventually contract a disease condition. Thus, for example, the term treatment includes the administration of an agent prior to or following the onset of a disease or disorder thereby preventing or removing all signs of the disease or disorder. As another example, administration of the agent after clinical manifestation of the disease to combat the symptoms of the disease comprises "treatment" of the disease.

[0038] As used herein, the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides. ESTs, chromosomes, cDNAs, mRNAs, and rRNAs are representative examples of molecules that may be referred to as nucleic acids.

[0039] As used herein, the term "pharmaceutical composition" refers to a mixture of at least one compound useful within the invention with other chemical components, such as carriers, stabilizers, diluents, adjuvants, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to: intra-tumoral, intravenous, intrapleural, oral, aerosol, parenteral, ophthalmic, pulmonary and topical administration.

[0040] The language "pharmaceutically acceptable carrier" includes a pharmaceutically acceptable salt, pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it may perform its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each salt or carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, and not injurious to the subject. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose,

ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; diluent; granulating agent; lubricant; binder; disintegrating agent; wetting agent; emulsifier; coloring agent; release agent; coating agent; sweetening agent; flavoring agent; perfuming agent; preservative; antioxidant; plasticizer; gelling agent; thickener; hardener; setting agent; suspending agent; surfactant; humectant; carrier; stabilizer; and other non-toxic compatible substances employed in pharmaceutical formulations, or any combination thereof. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound, and are physiologically acceptable to the subject. Supplementary active compounds may also be incorporated into the compositions.

[0041] As used herein, the term "effective amount" or "therapeutically effective amount" means the amount of the virus particle or infectious units generated from vector of the invention which is required to prevent the particular disease condition, or which reduces the severity of and/or ameliorates the disease condition or at least one symptom thereof or condition associated therewith.

[0042] A "subject" or "patient," as used therein, may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is a human.

[0043] It is to be appreciated that certain features of the invention which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination. Further, reference to values stated in ranges include each and every value within that range.

[0044] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is

merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

[0045] The present invention may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures and examples, which form a part of this disclosure. It is to be understood that this invention is not limited to the specific methods, applications, conditions or parameters described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed invention.

[0046] Methods of the invention

[0047] In one aspect, a method of treating a cancer in a subject in need thereof is disclosed herein. The method comprises administering to the subject an interferon type I (IFN-I) inhibiting agent comprising an mTOR inhibitor and an effective amount of Seneca Valley Virus (SVV) or SVV derivative, wherein the cancer is characterized by: (a) an expression level of anthrax toxin receptor 1 (ANTXR1) higher than an ANTXR1 reference value, and (b) an expression level of IFN-I higher than an IFN-I reference value.

[0048] In one aspect, another method of treating a cancer in a subject in need thereof is disclosed. The method comprises administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor and an effective amount of SVV or SVV derivative, wherein the cancer is characterized by an expression level of ANTXR1 higher than an ANTXR1 reference value, and wherein the IFN-I inhibiting agent reduces the expression level of IFN-I in the cancer thereby favoring replication of the SVV or the SVV derivative and reducing or eliminating (i.e. killing) the cancer. In on embodiment, the SVV or the SVV derivative induces antitumor immune response and triggers a switch from a cold tumor to a hot tumor. Cold tumors refer to

cancers that contain a low number of infiltrating T cells and are not recognized and do not provoke a strong response by the immune system, making them difficult to treat with current immunotherapies. Cancers that are classically immunologically cold include but are not limited to glioblastomas as well as ovarian, prostate, pancreatic, and most breast cancers. In contrast, immunologically hot tumors contain high levels of infiltrating T cells and more antigens, making them more recognizable by the immune system and more likely to trigger a strong immune response. Non limiting examples of cancers considered to be immunologically hot are bladder, head and neck, kidney, melanoma, and non–small cell lung cancers.

[0049] In another aspect, also disclosed herein is a method of predicting the efficacy of an SVV or an SVV derivative treatment of a cancer in a subject in need thereof. The method comprises determining the expression level of ANTXRl and the expression level of IFN-I in the cancer from the subject, wherein: (a) an expression level of ANTXRl higher than an ANTXRl reference value, and (b) an expression level of IFN-I higher than an IFN-I reference value are predictive that the treatment is effective, and wherein when the treatment is predicted to be effective, recommending treatment of the subject; wherein the treatment comprises administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor.

[0050] The treatment of cancer provided herein may include the treatment of solid tumors or the treatment of metastasis. Metastasis is a form of cancer wherein the transformed or malignant cells are traveling and spreading the cancer from one site to another. Such cancers include cancers of the skin, breast, brain, cervix, testes, etc. More particularly, cancers may include, but are not limited to the following organs or systems: cardiac, lung, gastrointestinal, genitourinary tract, liver, bone, nervous system, gynecological, hematologic, skin, and adrenal glands. More particularly, the methods herein can be used for treating gliomas (Schwannoma, glioblastoma, astrocytoma), neuroblastoma, pheochromocytoma, paraganlioma, meningioma, adrenalcortical carcinoma, kidney cancer, vascular cancer of various types, osteoblastic osteocarcinoma, prostate cancer, ovarian cancer, uterine leiomyomas, salivary gland cancer, choroid plexus carcinoma, mammary cancer, pancreatic cancer, colon cancer, and megakaryoblastic leukemia. Skin cancer includes malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, and psoriasis.

[0051] In some embodiments, the cancer treated by the presently disclosed methods comprises a triple negative breast cancer, a small cell lung cancer, a non-small cell lung cancer, a non-small cell squamous carcinoma, an adenocarcinoma, a glioblastoma, a skin cancer, a hepatocellular carcinoma, a colon cancer, a cervical cancer, an ovarian cancer, an endometrial cancer, a neuroendocrine cancer, a pancreatic cancer, a thyroid cancer, a kidney cancer, a bone cancer, an oesophagus cancer or a soft tissue cancer or any cancer expressing ANTXR1.

[0052] In some embodiments, the cancer treated by the presently disclosed methods comprises a cervical cancer or any cancer expressing ANTXR1.

[0053] Reference value or Control

[0054] The methods provided herein include comparing a measured expression level of ANTXR1 or IFN-I in a cancer from a subject to a reference value (i.e. the control amount) of expression of ANTXR1 or IFN-I.

[0055] In one embodiment, the reference level of expression of ANTXR1 or IFN-I may be obtained by measuring the expression level of ANTXR1 or IFN-I in a healthy subject and within the same cell type. Preferably, the healthy subject is a subject of similar age, gender and race and has never been diagnosed with any type of sever disease particularly any type of cancer.

[0056] In another embodiment, the reference value of expression of ANTXR1 or IFN-I is a value for expression of ANTXR1 or IFN-I that is accepted in the art. This reference value can be baseline value calculated for a group of subjects based on the average or mean values of ANTXR1 or IFN-I expression by applying standard statistically methods.

[0057] In one embodiment, the expression level is determined by a method selected from the group consisting of detecting mRNA of the gene, detecting a protein encoded by the gene, and detecting a biological activity of the protein encoded by the gene.

[0058] In certain aspects of the present invention, the expression level of ANTXR1 or IFN-I is determined in a cancerous sample from a subject. The sample preferably includes tumor cells, any fluid from the surrounding of tumor cells (e.g. leukemic blood, or tumor tissue) or any fluid that is in physiological contact or proximity with the tumor, or any other body fluid in addition to those recited herein should also be considered to be included herein.

[0059] Methods of Measurement

[0060] Any method known to those in the art can be employed for determining the expression level of ANTXR1, IFN-I and/or other biomarkers at the transcriptional or

translational level. For example, a microarray can be used. Microarrays are known in the art and consist of a surface to which probes that correspond in sequence to gene products (e.g. mRNAs, polypeptides, fragments thereof etc.) can be specifically hybridized or bound to a known position. To detect at least one gene of interest, a hybridization sample is formed by contacting the test sample with at least one nucleic acid probe. A preferred probe for detecting ANTXR1 and/or IFN-I is a labeled nucleic acid probe capable of hybridizing to ANTXR1 and/or IFN-I mRNA(s). The nucleic acid probe can be, for example, a full-length nucleic acid molecule, or a portion thereof, such as an oligonucleotide of at least 10, 15, or 20 nucleotides in length and sufficient to specifically hybridize under stringent conditions to the appropriate target. The hybridization sample is maintained under conditions which are sufficient to allow specific hybridization of the nucleic acid probe to a target of interest. Specific hybridization can be performed under high stringency conditions or moderate stringency conditions, as appropriate. In a preferred embodiment, the hybridization conditions for specific hybridization are high stringency. Specific hybridization, if present, is then detected using standard methods. If specific hybridization occurs between the nucleic acid probe and a gene in the test sample, the sequence that is present in the nucleic acid probe is also present in the mRNA of the subject. More than one nucleic acid probe can also be used. Hybridization intensity data detected by the scanner are automatically acquired and processed by the Affymetrix Microarray Suite (MASS) software. Raw data is normalized to expression levels using a target intensity of 150. An alternate method to measure mRNA expression profiles of a small number of different genes is by e.g. either classical TaqMan® Gene Expression Assays or TaqMan® Low Density Array—micro fluidic cards (Applied Biosystems) and Nanostring technology. Particularly, this invention preferably utilizes a qPCR system. Non-limiting examples include commercial kits such as the PrimePCRPathways® commercially available from Bio-rad (Berkley, California).

[0061] The transcriptional state of a sample, particularly mRNAs, may also be measured by other nucleic acid expression technologies known in the art. mRNA can be isolated from the sample using any method known to those in the art. Non-limiting examples include commercial kits, such as the RNeasy® commercially available from Qiagen (Netherlands) or the Mini Kit the TRI Reagent® commercially available from Molecular Research Center, Inc. (Cincinnati, Ohio), can be used to isolate RNA. Generally, the isolated mRNA may be amplified using methods known in the art. Amplification systems utilizing, for example, PCR or RT-PCR

methodologies are known to those skilled in the art. For a general overview of amplification technology, see, for example, Dieffenbach *et al.*, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1995).

[0062] Another accurate method for profiling mRNA expression can the use of Next Generation Sequencing (NGS) including first, second, third as well as subsequent Next Generations Sequencing technologies.

[0063] In other aspects provided herein, determining the amount or detecting the biological activity of a peptide, polypeptide can be achieved by all known means in the art for determining the amount of a peptide or polypeptide in a sample. These means comprise immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Such assays will develop a signal which is indicative for the presence or absence of the peptide or polypeptide. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse- proportional) to the amount of polypeptide present in a sample. Further suitable methods comprise measuring a physical or chemical property specific for the peptide or polypeptide such as its precise molecular mass or NMR spectrum. These methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass- spectrometers, NMR- analyzers, HPLC, FPLC, or chromatography devices. Further, methods include, Western blots, micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on Elecsys™ analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-Hitachi™ analyzers), and latex agglutination assays (available for example on Roche-Hitachi™ analyzers).

[0064] In some embodiments, for the various methods disclosed herein, the expression level of ANTXR1 is determined based on the level of an ANTXR1 mRNA or an ANTXR1 protein and the expression level of IFN-I is determined based on the level of an IFN-I biomarker mRNA or an IFN-I biomarker protein. In one embodiment, the IFN-I biomarker mRNA or IFN-I biomarker protein is at least one mRNA or protein selected from the group consisting of IFI35, IFN-α, IFN-β, IFN-κ, IFN-δ, IFN-ε, IFN-τ, IFN-ω, and IFN-ζ, ADAR, IRF9, IFITM3, IFITM2, USP18, LOC144383, EGR1, IFI6, GBP2, HLA-A, HLA-B, HLA-C, HLA-F, HLA-G, IRF8, IFI27, IFI35, IFIT2, IFIT1, IFIT3, IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNAR1, IFNAR2, IFNB1, IRF1, IRF2, IRF3,

IRF4, IRF5, IRF6, IRF7, ISG20, JAK1, MX1, MX2, OAS1, OAS2, OAS3, IP6K2, XAF1, PSMB8, PTPN1, PTPN6, RNASEL, HLA-K, STAT1, STAT2, TYK2, HLA-B, IFITM1, OASL, SOCS1, SOCS3 and ISG15.

[0065] Combination Therapies

[0066] The compositions and methods for treating a cancer in a subject using SVV or SVV derivatives described herein may be useful when combined with at least one additional compound useful for treating cancer. The additional compound may comprise a commercially available compound, known to treat, prevent, or reduce the symptoms of cancer and/or metastasis.

[0067] In one aspect, the pharmaceutical composition disclosed herein comprises an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier. The pharmaceutical composition may be used in combination with a therapeutic agent such as an anti-tumor agent, including but not limited to a chemotherapeutic agent, an anti-cell proliferation agent or any combination thereof. For example, any conventional chemotherapeutic agents of the following non-limiting exemplary classes are included in the invention: alkylating agents; nitrosoureas; antimetabolites; antitumor antibiotics; plant alkyloids; taxanes; hormonal agents; and miscellaneous agents. In another aspect, the pharmaceutical composition disclosed herein may be used in combination with a radiation therapy.

[0068] Most alkylating agents are cell cycle non-specific. In specific aspects, they stop tumor growth by cross-linking guanine bases in DNA double-helix strands. Non-limiting examples include busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mechlorethamine hydrochloride, melphalan, procarbazine, thiotepa, and uracil mustard.

[0069] Anti-metabolites prevent incorporation of bases into DNA during the synthesis (S) phase of the cell cycle, prohibiting normal development and division. Non-limiting examples of antimetabolites include drugs such as 5-fluorouracil, 6-mercaptopurine, capecitabine, cytosine arabinoside, floxuridine, fludarabine, gemcitabine, methotrexate, and thioguanine.

[0070] Antitumor antibiotics generally prevent cell division by interfering with enzymes needed for cell division or by altering the membranes that surround cells. Included in this class are the anthracyclines, such as doxorubicin, which act to prevent cell division by disrupting the structure of the DNA and terminate its function. These agents are cell cycle non-

specific. Non-limiting examples of antitumor antibiotics include aclacinomycin, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carubicin, caminomycin, carzinophilin, chromomycin, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mitoxantrone, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin.

[0071] Plant alkaloids inhibit or stop mitosis or inhibit enzymes that prevent cells from making proteins needed for cell growth. Frequently used plant alkaloids include vinblastine, vincristine, vindesine, and vinorelbine. However, the invention should not be construed as being limited solely to these plant alkaloids.

[0072] The taxanes affect cell structures called microtubules that are important in cellular functions. In normal cell growth, microtubules are formed when a cell starts dividing, but once the cell stops dividing, the microtubules are disassembled or destroyed. Taxanes prohibit the microtubules from breaking down such that the cancer cells become so clogged with microtubules that they cannot grow and divide. Non-limiting exemplary taxanes include paclitaxel and docetaxel.

[0073] Hormonal agents and hormone-like drugs are utilized for certain types of cancer, including, for example, leukemia, lymphoma, and multiple myeloma. They are often employed with other types of chemotherapy drugs to enhance their effectiveness. Sex hormones are used to alter the action or production of female or male hormones and are used to slow the growth of breast, prostate, and endometrial cancers. Inhibiting the production (aromatase inhibitors) or action (tamoxifen) of these hormones can often be used as an adjunct to therapy. Some other tumors are also hormone dependent. Tamoxifen is a non-limiting example of a hormonal agent that interferes with the activity of estrogen, which promotes the growth of breast cancer cells.

[0074] Miscellaneous agents include chemotherapeutics such as bleomycin, hydroxyurea, L-asparaginase, and procarbazine.

[0075] Other examples of chemotherapeutic agents include, but are not limited to, the following and their pharmaceutically acceptable salts, acids and derivatives: MEK inhibitors, such as but not limited to, refametinib, selumetinib, trametinib or cobimetinib; nitrogen mustards

such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatrexate; defofamine; demecolcine; diaziquone; eflornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; polysaccharide-K (PSK); razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2′,2″-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOLO, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; and capecitabine.

[0076] An anti-cell proliferation agent can further be defined as an apoptosis-inducing agent or a cytotoxic agent. The apoptosis-inducing agent may be a granzyme, a Bcl-2 family member, cytochrome C, a caspase, or a combination thereof. Exemplary granzymes include granzyme A, granzyme B, granzyme C, granzyme D, granzyme E, granzyme F, granzyme G, granzyme H, granzyme I, granzyme J, granzyme K, granzyme L, granzyme M, granzyme N, or a combination thereof. In other specific aspects, the Bcl-2 family member is, for example, Bax, Bak, Bcl-Xs, Bad, Bid, Bik, Hrk, Bok, or a combination thereof.

[0077] In additional aspects, the caspase is caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, caspase-11, caspase-12,

caspase-13, caspase-14, or a combination thereof. In specific aspects, the cytotoxic agent is TNF-α, gelonin, Prodigiosin, a ribosome-inhibiting protein (RIP), *Pseudomonas* exotoxin, *Clostridium difficile* Toxin B, *Helicobacter pylori* VacA, *Yersinia enterocolitica* YopT, Violacein, diethylenetriaminepentaacetic acid, irofulven, Diptheria Toxin, mitogillin, ricin, botulinum toxin, cholera toxin, saporin 6, or a combination thereof.

[0078] An immunotherapeutic agent may be, but is not limited to, an interleukin-2 or other cytokine, an inhibitor of programmed cell death protein 1 (PD-1) signaling such as a monoclonal antibody that binds to PD-1, Ipilimumab. The immunotherapeutic agent can also block cytotoxic T lymphocytes associated antigen A-4 (CTLA-4) signaling and it can also relate to cancer vaccines and dendritic cell-based therapies.

[0079] In one embodiment the subject suffering from cancer is administered at least one anti-cancer therapeutic agent selected from the group consisting of: a checkpoint inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a cytokine, a growth factor, a photosensitizing agent, a toxin, a siRNA molecule, a signaling modulator, an anti-cancer antibiotic, an anti-cancer antibody, an angiogenesis inhibitor, a chemotherapeutic compound, anti-metastatic compound, an immunotherapeutic compound, a CAR therapy, a dendritic cell-based therapy, a cancer vaccine, an oncolytic virus, an engineered anti-cancer virus or virus derivative and a combination of any thereof. In one embodiment, the least one anti-cancer therapeutic agent is administered formerly, simultaneously or subsequently to the administering of the SVV or SVV derivative.

[0080] In one embodiment, the subject is administered an IFN-I inhibiting agent. The IFN-I inhibiting agent used herein encompasses any agent known in the art for inhibiting, suppressing or reducing partially or fully and temporarily or permanently IFN type I pathway. In some embodiments, the inhibition effect of the IFN-I inhibiting agent can be reversible. In other embodiments, the inhibition of the IFN-I is reversed.

[0081] The inhibiting agent comprises siRNA, ribozyme, an antisense molecule, an aptamer, a peptidomimetic, a small molecule, an mTOR inhibitor, a histone deacetylase (HDAC) inhibitor, a Janus kinase (JAK) inhibitor, an IFN inhibitor, an IFN antibody, an IFN-α Receptor 1 antibody, an IFN-α Receptor 2 antibody and viral peptide and a combination of any thereof. The viral peptide can be, but not limited to, NS1 protein from an Influenza virus or NS2B3 protease complex from dengue virus.

[0082] The mTOR pathway and its inhibition are known to be implicated in various diseases such as cancer. Rapamycin is a natural product produced by the bacterium *Streptomyces hygroscopicus* that can inhibit mTOR through association with its intracellular receptor FK-506 binding protein 12 (FKBP12). The FKBP12-rapamycin complex binds directly to the FKBP12-rapamycin binding domain of mTOR. mTOR functions as a catalytic subunit for two distinct molecular complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is composed of regulatory associated protein of mTOR (Raptor) and mammalian LST8/G-protein β-subunit like protein (mLST8/GβL). This complex functions as a nutrient/energy/redox sensor and plays a role in regulating protein synthesis. The activity of mTORC1 is stimulated by insulin, growth factors, serum, phosphatidic acid, amino acids (particularly leucine) and oxidative stress (Hay and Sonenberg, Genes Dev. 18(16):1926-1945, 2004; Wullschleger et al., Cell 124(3):471-484). In contrast, mTORC1 is known to be inhibited by low nutrient levels, growth factor deprivation, reductive stress, caffeine, rapamycin, farnesylthiosalicylic acid and curcumin (Beevers et al., Int. J. Cancer 119(4):757-764, 2006; McMahon et al., Mol. Endocrinol. 19(1):175-183). The components of mTORC2 are rapamycin-insensitive companion of mTOR (Rictor), GβL, mammalian stress-activated protein kinase interacting protein 1 and mTOR. mTORC2 has been shown to function as an important regulator of the cytoskeleton through its stimulation of F-actin stress fibers, paxillin, RhoA, Rac1, Cdc42 and protein kinase C alpha (Sarbassov et al., Curr. Biol. 14(14): 1296-302, 2004; Sarbassov et al., Science 307(5712): 1098-101, 2005). Unlike mTORC1, mTORC2 is not sensitive to rapamycin.

[0083] A number of mTOR inhibitors are known in the art and have potent immunosuppressive and anti-tumor activities. Inhibitors of mTOR, such as rapamycin or rapamycin analogs or derivatives, are known to exhibit immunosuppressive and anti-proliferative properties. Other mTOR inhibitors include everolimus, tacrolimus, zotarolimus (ABT-578), pimecrolimus, biolimus, FK-506, PP242 (2-(4-Amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-1H-indol-5-ol), Ku-0063794 (re1-5-[2-[(2R,6S)-2,6-Dimethyl-4-morpholinyl]-4-(4-morpholinyl)pyrido[2,3-d]pyrimidin-7-yl]-2-methoxybenzenemethanol), PI-103 (3-(4-(4-Morpholinyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-2-yl)phenol), PKI-179 (N-[4-[4-(4-Morpholinyl)-6-(3-oxa-8-azabicyclo[3.2.1]oct-8-yl)-1,3,5-triazin-2-yl]phenyl]-N′-4-pyridinylurea hydrochloride), AZD8055 (5-[2,4-Bis[(3S)-3-methyl-4-morpholinyl]pyrido[2,3-

d]pyrimidin-7-yl]-2-methoxybenzenemethanol), WYE-132/WYE-125132 (1-{4-[1-(1,4-Dioxa-spiro[4.5]dec-8-yl)-4-(8-oxa-3-aza-bicyclo[3.2.1]oct-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl]-phenyl}-3-methyl-urea), WYE-23 (4-{6-[4-(3-Cyclopropyl-ureido)-phenyl]-4-morpholin-4-yl-pyrazolo[3,4-d]pyrimidin-1-yl}-piperidine-1-carboxylic acid methyl ester), WYE-28 (4-(6-{4-[3-(4-Hydroxymethyl-phenyl)-ureido]-phenyl}-4-morpholin-4-yl-pyrazolo[3,4-d]pyrimidin-1-yl)-piperidine-1-carboxylic acid methyl ester), WYE-354 (4-[6-[4-[(Methoxycarbonyl)amino]phenyl]-4-(4-morpholinyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-1piperidinecarboxylic acid methyl ester), C20-methallylrapamycin and C16-(S)-butylsulfonamidorapamycin, C16-(S)-3-methylindolerapamycin (C16-iRap), C16-(S)-7-methylindolerapamycin (AP21967/C16-AiRap), CCI-779 (temsirolimus), RAD001(40-O-(2-hydroxyethyl)-rapamycin), AP-23575, AP-23675, AP-23573, 20-thiarapamycin, 15-deoxo-19-sulfoxylrapamycin, WYE-592, ILS-920, (3S,6R,7E,9R,10R,12R,14S,15E,17E,19E,21S,23S,26R,27R,34aS)-9,10,12,13,14,2-1,22,23,24,25,26,27,32,33,34,34a-Hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(-1S,3R,4R)-3-methoxy-4-tetrazol-1-yl)cyclohexyl]-1-methylethyl]-10,21-dime-t-hoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c] [1,4]oxaazac-yc-lohentriacontine-1,5,11,28,29(4H,6H,31H)-pentone) 23,27-Epoxy-3H pyrido [2,1-c] [1,4]oxaazacyclohentriacontine-1,5,11,28,29(4H,6H,31H)-pentone (U.S. Pat. No. 6,015,815), U.S. Pat. No. 6,329,386, U.S. Publication 2003/129215, U.S. Publication 2002/123505, A-94507, Deforolimus, AP-23675, AP-23841, Zotarolimus, CCI779/Temsirolimus, RAD-001/Everolimus, 7-epi-rapamycin, 7-thiomethyl-rapamycin, 7-epi-trimethoxy-rapamycin, 2-desmethyl-rapamycin, and 42-O-(2-hydroxy)ethyl-rapamycin, AP-23841, 7-epi-rapamycin, 7-thiomethyl-rapamycin, 7-epi-trimethoxyphenyl-rapamycin, 7-epi-thiomethyl-rapamycin, 7-demethoxy-rapamycin, 32-demethoxy-rapamycin, 2-desmethyl-rapamycin, 42-O-(2-hydroxy)ethyl rapamycin, ridaforolimus, ABI-009, MK8669, TOP216, TAFA93, TORISEL™ (prodrug), CERTICAN™, Ku-0063794, PP30, Torin1, Torin2, ECO371, AP23102, AP23573, AP23464, AP23841; 40-(2-hydroxyethyl)rapamycin, 40-[3-hydroxy(hydroxymethyl) methylpropanoate]-rapamycin (also called CC1779), 32-deoxorapamycin, and 16-pentynyloxy-32(S)-dihydrorapanycin. Non-rapamycin analog mTOR inhibiting compounds include, but are not limited to, LY294002, wortmannin, quercetin, myricentin, staurosporine, and ATP competitive inhibitors.

[0084] In some embodiments, the disclosed mTOR inhibitor inhibits at least one of mTORC1 and mTORC2. In other embodiments, the disclosed mTOR inhibitor is Torin 1 or Torin 2.

[0085] A large number of HDAC inhibitors are known and used in the art. The most common HDAC inhibitors bind to the zinc-containing catalytic domain of the HDACs. These HDAC inhibitors can be classified into several groupings named according to their chemical structure and the chemical moiety that binds to the zinc ion. Some examples include, but are not limited to, hydroxamic acids or hydroxamates (such as Trichostatin A or Vorinostat/SAHA (FDA approved)), aminobenzamides Entinostat (MS-275), Tacedinaline (CI994), and Mocetinostat (MGCD0103), cyclic peptides (Apicidin, Romidepsin (FDA approved)), cyclic tetrapeptides or epoxyketones (such as Trapoxin B), depsipeptides, benzamides, electrophilic ketones, and carboxylic aliphatic acid compounds (such as butyrate, phenylbutyrate, valproate and valproic acid). Other HDAC inhibitors include, but are not limited not, Belinostat (PXD101), LAQ824, and Panobinostat (LBH589). Examples of HDCA inhibitors in clinical trials include Panobinostat (LBH-589), Belinostat (PXD101), Entinostat (MS275), Mocetinostat (MGCD01030), Givinostat (ITF2357), Practinostat (SB939), Chidamide (CS055/HBI-8000), Quisinostat (JNJ-26481585), Abexinostat (PCI-24781), CHR-3996 and AR-Z2. In one embodiment, the HDAC inhibitor is Trichostatin A (TSA).

[0086] JAK inhibitors (also referred as JAK/SAT inhibitors) inhibit the activity of one or more of the Janus kinase family of enzymes (e.g. JAK1, JAK2, JAK3 and/or TYK2), thereby interfering with the JAK-STAT signaling pathway. Various JAK inhibitors are known and used in the art for the treatment of inflammatory diseases or cancer. Non-limiting examples of JAK inhibitors are FDA approved compounds including Ruxolitinib (Jakafi/Jakavi), Tofacitinib (Jakvinus, formerly known as tasocitinib and CP-690550), Oclacitinib (Apoquel), Baricitinib (Olumiant, LY3009104), Decernotinib (VX-509). Other JAK inhibitors are under clinical trials and/or used as experimental drugs. These include for instance Filgotinib (G-146034, GLPG-0634), Cerdulatinib (PRT062070), Gandotinib (LY-2784544), Lestaurtinib (CEP-701), Momelotinib (GS-0387, CYT-387), Pacritinib (SB1518), PF-04965842, Upadacitinib (ABT-494), Peficitinib (ASP015K, JNJ-54781532), Fedratinib (SAR302503), Cucurbitacin I, CHZ868, ABT-494, dimethyl fumarate (DMF, Tecfidera), GLPG0634, and CEP-33779. In one

embodiment, the JAK/STAT inhibitor is staurosporine (STS; antibiotic AM-2282) which is an inhibitor of protein kinase C (PKC).

[0087] In one embodiment, the subject is further administered at least one IFN-I inhibiting agent selected from the group consisting of: HDAC inhibitor, JAK/STAT inhibitor, IFN inhibitor, IFN antibody, IFN-α Receptor 1 antibody, IFN-α Receptor 2 antibody and viral peptide and a combination of any thereof. In another embodiment, the at least one IFN-I inhibiting agent is administered formerly, simultaneously or subsequently to the administering of the SVV or SVV derivative. In some embodiments, the at least one IFN-I inhibiting agent is subsequently removed once the SVV has replicated in the tumor cells and before the addition of an anti-cancer therapeutic agent (e.g. checkpoint inhibitor).

[0088] In one embodiment, the anti-cancer therapeutic agent is administered formerly, simultaneously or subsequently to the administering of the at least one IFN-I inhibiting agent. In one embodiment, the anti-cancer therapeutic agent is administered subsequently to the administering of the at least one IFN-I inhibiting agent. In another embodiment, the anti-cancer therapeutic agent is administered subsequently to the administering of the at least one IFN-I inhibiting agent and the SVV or SVV derivative.

[0089] In one embodiment the SVV or SVV derivative treatment is preceded by the administration of IFN-I inhibiting agent. In one embodiment, once SVV replication and cancer cells death are confirmed, the administration of IFN-I inhibiting agent is terminated. For instance, cancer cells can be treated with an IFN-I inhibitor, (e.g. (5-(tetradecyloxy)-2-furoic acid), acetyl-CoA carboxylase inhibitor: TOFA), 24 hours before SVV treatment and then both treatments can be pursued for several weeks until robust SVV replication is observed and markers of cell death are detected. Then the treatment with IFN-I inhibiting agent can be terminated and an anti-cancer therapeutic agent (such as but not limited to a checkpoint inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor or a CTLA-4 inhibitor) can be initiated. Upon SVV replication, various nucleic acids and cellular debris are generated which can trigger the activation of an influx of immune cells (e.g. T-cells, NK, cells, APCs, etc.) to proceed in cancer cells' inhibition, reduction and/or elimination/killing. This process of immune response is enhanced further by the termination of IFN-I inhibition.

[0090] Pharmaceutical Compositions and Formulations.

[0091] Provided herein is a pharmaceutical composition for treating a cancer in a subject in need thereof. The pharmaceutical composition comprises an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier.

[0092] Also provided herein is the use of a pharmaceutical composition for treating for treating and/or in the manufacture of a drug for treating cancer in a subjct in need thereof. The pharmaceutical composition comprises an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier.

[0093] Such a pharmaceutical composition is in a form suitable for administration to a subject, or the pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The various components of the pharmaceutical composition may be present in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

[0094] In an embodiment provided herein, the pharmaceutical composition useful for practicing the method of the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical composition useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 500 mg/kg/day. The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0095] Pharmaceutical compositions that are useful in the methods of the invention may be suitably developed for inhalational, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intravenous or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration is readily apparent to the skilled artisan and depends upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

[0096] The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit. In some embodiments, the SVV or derivative thereof can be formulated in a natural capsid, a modified capsid, as a naked RNA, or encapsulated in a protective coat.

[0097] The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

[0098] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions suitable for ethical administration to humans, it is understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs. In one embodiment, the subject is a human or a non-human mammal such as but not limited to an equine, an ovine, a bovine, a porcine, a canine, a feline and a murine. In one embodiment, the subject is a human.

[0099] In one embodiment, the compositions are formulated using one or more pharmaceutically acceptable excipients or carriers. In one aspect a pharmaceutical composition is disclosed for treating a cancer in a subject. The pharmaceutical composition comprises an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier. Pharmaceutically acceptable carriers, which are useful, include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically

acceptable salt solutions such as phosphates and salts of organic acids. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The 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. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

[00100] Formulations may be employed in admixtures with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, e.g., other analgesic agents.

[00101] The disclosed composition may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of exposure to contaminants in the environment. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

[00102] The composition may include an antioxidant and a chelating agent which inhibit the degradation of the compound. Preferred antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the

composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition which may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefore as would be known to those skilled in the art.

[00103] Administration/Dosing

[00104] The regimen of administration may affect what constitutes an effective amount. For example, the therapeutic formulations may be administered to the patient subject either prior to or after a surgical intervention related to cancer, or shortly after the patient was diagnosed with cancer. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

[00105] In general, SVV is administered in an amount of between 10^7 and 1×10^{11} vp/kg. The exact dosage to be administered depends on a variety of factors including the age, weight, and sex of the patient, and the size and severity of the tumor being treated.

[00106] SVV is typically administered at a therapeutically effective dose. A therapeutically effective dose refers to that amount of the virus that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of viruses can be determined by standard procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population of animals or cells; for viruses, the dose is in units of vp/kg) and the ED50 (the dose, vp/kg, therapeutically effective in 50% of the population of animals or cells), or the TC10 (the therapeutic concentration or dose allowing inhibition of 50% of tumor cells and can be related to PFU) or the EC50 (the effective concentration, vp/cell, in 50% of the population of animals or cells). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD50 and ED50 or EC50. The dosage of viruses lies preferably within a range of circulating

concentrations that include the ED50 or EC50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed-and the route of administration utilized.

[00107] The SVV may be present in the composition in multidose and single dosage amounts, including, but not limited to between or between about 1×10^5 and 1×10^{12} pfu, 1×10^6 to 1×10^{10} pfu, or 1×10^7 to 1×10^{10} pfu, each inclusive, such as at least, or about at least 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10}, 1×10^{11}, or 1×10^{12} pfu.

[00108] The volume of the composition can be any volume, and can be for single or multiple dosage administration, including, but not limited to, from or from about 0.01 mL to 100 mL, 0.1 mL to 100 mL, 1 mL to 100 mL, 10 mL to 100 mL, 0.01 mL to 10 mL, 0.1 mL to 10 mL, 1 mL to 10 mL, 0.02 mL to 20 mL, 0.05 mL to 5 mL, 0.5 mL to 50 mL, or 0.5 mL to 5 mL, each inclusive.

[00109] The infectivity of the SVV can be manifested, such as by increased titer or half-life of the oncolytic virus when exposed to a bodily fluid, such as blood or serum. Infectivity can be increased by any amount, including, but not limited to, at least 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.5-fold, 3-fold, 4-fold, 5-fold. 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold.

[00110] Administration of the compositions of the present invention to a patient subject, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat cancer in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is from about 0.01 and 50 mg/kg of body weight/per day.

[00111] The compound can be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose is readily apparent to the skilled artisan and depends upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, and the type and age of the animal. Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. A medical doctor, e.g., physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[00112] In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of cancer in a patient.

[00113] Routes of Administration

[00114] One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route.

[00115] Routes of administration of the disclosed compositions include inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (e.g., sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (e.g., trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration. Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein. In one embodiment, the SVV or SVV derivative treatment comprises an administration route selected from the group consisting of inhalation, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intra-hepatic arterial, intrapleural, intrathecal, intra-tumoral, intravenal and any combination thereof.

[00116] In yet another aspect, also provided herein is a kit for determining a predisposition of an efficacious response to an SVV or an SVV derivative based treatment of a cancer in a subject, wherein the treatment comprises an mTOR inhibitor. The disclosed kit comprises a reagent for determining the expression level of ANTXRl and a reagent for determining the expression level of IFN-I in the cancer from the subject.

[00117] Kit

[00118] The invention includes a set of preferred oligomers or antibodies, either labeled (e.g., fluorescer, quencher, etc.) or unlabeled, that are useful for the detection of at least ANTXR1 and/or IFN-I.

[00119] In certain embodiments, a kit is provided. Commercially available kits for use in these methods are, in view of this specification, known to those of skill in the art. In general,

kits will comprise a detection reagent that is suitable for detecting the presence of a polypeptide or nucleic acid, or mRNA of interest.

[00120] In another embodiment, there is a panel of probe sets or antibodies. Preferred probe sets are designed to detect the expression level of ANTXR1 and/or IFN-I and provide information about the efficacy of an SVV or an SVV derivative based cancer treatment. Probe sets are particularly useful because they are smaller and cheaper than probe sets that are intended to detect as many polynucleotides or peptides as possible in a particular genome. As provided herein, the probe sets are targeted at the detection of polynucleotides or polypeptides that are informative about ANTXR1 and/or IFN-I in cancer cells or tissues. Probe sets may also comprise a large or small number of probes that detect polynucleotides or peptides that are not informative about cancer. Such probes are useful as controls and for normalization (e.g., spiked-in markers). Probe sets may be a dry mixture or a mixture in solution. In some embodiments, probe sets can be affixed to a solid substrate to form an array of probes. The probes may be antibodies, or nucleic acids (e.g., DNA, RNA, chemically modified forms of DNA and RNA), LNAs (Locked nucleic acids), or PNAs (Peptide nucleic acids), or any other polymeric compound capable of specifically interacting with the peptides or nucleic acid sequences of interest.

[00121] It is contemplated that kits may be designed for isolating and/or detecting peptides (e.g. ANTXR1, know cancer markers, immune activators or apoptotic proteins) or nucleic acid sequences in essentially any sample (e.g., leukemic blood, tumor cells, tumor tissue, etc...), and a wide variety of reagents and methods are, in view of this specification, known in the art.

[00122] In further embodiments a kit is provided for treating or ameliorating a cancer, as described elsewhere herein wherein the kit comprises: a) a compound or compositions as described herein; and b) an additional agent or therapy as described herein. The kit can further include instructions or a label for using the kit to treat or ameliorate the cancer. In yet other embodiments, the invention extends to kits assays for a given cancer (such as, but not limited to, small-cell lung cancer or triple negative breast cancer), as described herein. Such kits may, for example, contain the reagents from PCR or other nucleic acid hybridization technology (microarrays) or reagents for immunologically based detection techniques (e.g., ELISpot, ELISA).

ILLUSTRATIVE EMBODIMENTS

[00123] Provided here are illustrative embodiments of the disclosed technology. These embodiments are illustrative only and do not limit the scope of the present disclosure or of the claims attached

[00124] Embodiment 1. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an interferon type I (IFN-I) inhibiting agent comprising an mTOR inhibitor and an effective amount of Seneca Valley Virus (SVV) or SVV derivative, wherein the cancer is characterized by:

a. an expression level of anthrax toxin receptor 1 (ANTXRl) higher than an ANTXRl reference value, and

b. an expression level of IFN-I higher than an IFN-I reference value.

[00125] Embodiment 2. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor and an effective amount of SVV or SVV derivative, wherein the cancer is characterized by an expression level of ANTXRl higher than an ANTXRl reference value, and wherein the IFN-I inhibiting agent reduces the expression level of IFN-I in the cancer thereby favoring replication of the SVV or the SVV derivative and reducing or eliminating the cancer.

[00126] Embodiment 3. A method of predicting the efficacy of a Seneca Valley Virus (SVV) treatment, or an SVV derivative treatment of a cancer in a subject in need thereof, the method comprising determining the expression level of ANTXRl and the expression level of IFN-I in the cancer from the subject, wherein:

c. an expression level of ANTXRl higher than an ANTXRl reference value, and

d. an expression level of IFN-I higher than an IFN-I reference value

are predictive that the treatment is effective, and wherein when the treatment is predicted to be effective, recommending treatment of the subject; and

wherein the treatment comprises administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor.

[00127] Embodiment 4. A pharmaceutical composition for treating a cancer in a subject in need thereof, the pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier.

[00128] Embodiment 5. The use of a pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative for treating a cancer in a patient in need thereof.

[00129] Embodiment 6. The use of a pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative in the manufacture of a drug for treating a cancer in a patient in need thereof.

[00130] Embodiment 7. The method of any one of embodiments 1-3, wherein the expression level of ANTXR1 is determined based on the level of an ANTXR1 mRNA or an ANTXR1 protein.

[00131] Embodiment 8. The method of any one of embodiments 1-3, wherein the expression level of IFN-I is determined based on the level of an IFN-I biomarker mRNA or an IFN-I biomarker protein.

[00132] Embodiment 9. The method or pharmaceutical composition of any one of embodiments 1-8, wherein the subject is administered at least one anti-cancer therapeutic agent selected from the group consisting of: a checkpoint inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a cytokine, a growth factor, a photosensitizing agent, a toxin, a siRNA molecule, a signaling modulator, an anti-cancer antibiotic, an anti-cancer antibody, an angiogenesis inhibitor, a chemotherapeutic compound, anti-metastatic compound, an immunotherapeutic compound, a CAR therapy, a dendritic cell-based therapy, a cancer vaccine, an oncolytic virus, an engineered anti-cancer virus or virus derivative and a combination of any thereof.

[00133] Embodiment 10. The method or pharmaceutical composition of embodiment 9, wherein the at least one anti-cancer therapeutic agent is administered formerly, simultaneously or subsequently to the administering of the SVV.

[00134] Embodiment 11. The method or pharmaceutical composition of any one of embodiments 1-10, wherein the mTOR inhibitor inhibits at least one of mTORC1 and mTORC2.

[00135] Embodiment 12. The method or pharmaceutical composition of embodiment 11, wherein the mTOR inhibitor is Torin 2.

[00136] Embodiment 13. The method or pharmaceutical composition of any one of embodiments 1-12, wherein the cancer comprises a triple negative breast cancer, small cell lung cancer, a non-small squamous cell carcinoma, a skin cancer, a hepatocellular carcinoma, a colon

cancer, a cervical cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a thyroid cancer, a kidney cancer, a bone cancer, an oesophagus cancer, a soft tissue cancer or any cancer expressing ANTXR1.

[00137] Embodiment 14. The method or pharmaceutical composition of embodiment 13, wherein the cancer comprises a cervical cancer.

[00138] Embodiment 15. The method or pharmaceutical composition of any one of embodiments 1-14, wherein the subject is further administered at least one additional IFN-I inhibiting agent selected from the group consisting of: HDAC inhibitor, JAK/STAT inhibitor, IFN inhibitor, IFN antibody, IFN-α Receptor 1 antibody, IFN-α Receptor 2 antibody and viral peptide and a combination of any thereof.

[00139] Embodiment 16. The method of embodiment 15, wherein the HDAC inhibitor is Trichostatin A.

[00140] Embodiment 17. The method of any one of embodiments 15-16, wherein the JAK/STAT inhibitor is staurosporine.

[00141] Embodiment 18. A Seneca Valley Virus (SVV) or SVV derivative in combination with IFN-I inhibiting agent comprising an mTOR inhibitor for use in the manufacture of a medicament for treatment of a cancer, wherein the cancer is characterized by an expression level of anthrax toxin receptor 1 (ANTXRl) higher than an ANTXRl reference value, and wherein the IFN-I inhibiting agent reduces the expression level of IFN-I in the cancer thereby favoring replication of the SVV or the SVV derivative and reducing or eliminating the cancer.

EXAMPLES

[00142] The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

[00143] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working

examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Materials and Methods

[00144] Cells and viruses. Cells were maintained in DMEM supplemented with 10% fetal bovine serum (Per.C6) or 10% bovine serum (HeLa), 2mM L-glutamine (Invitrogen), and 1% penicillin-streptomycin. Viral stocks were produced by growth in Per.C6 cells as previously described (23).

[00145] Cytokines and Inhibitors. IFN-α and Trichostatin A were purchased from Sigma, Inc. (St. Louis, MO). Staurosporine was purchased from Cell Signaling Technologies (Cambridge, MA); Torin 2 was from Tocris Bioscience (Bristol, UK). All compounds were dissolved in DMSO.

[00146] Virus infections. Infections were performed in 35mm dishes. Cells were counted at the time of infection and viral stocks were diluted in PBS+0.01%BSA (Sigma, St. Louis, MO) for infections at the appropriate MOI. One hundred microliters of diluted virus were used to infect each plate at 37oC for 45 min with rocking. Cells were then washed twice with PBS and covered with 1mL of appropriate media; samples taken at this time are called 0 time point. Mock infected cells were treated with PBS+0.01%BSA instead of virus. Three independent infections were done.

[00147] Plaque assays. Samples for virus titration were produced by harvesting cells and medium, followed by three cycles of freezing and thawing and then by centrifugation at 5000xg for 5 min. All titrations were done on monolayers of Per.C6 cells in 35 mm plates. Ten-fold serial dilutions of virus were made in PBS+0.1 mg/ml BSA (Sigma) and 0.1mL of each dilution was added per well. Plates were incubated at 37oC for 45min with rocking and overlay was then added. A two-overlay system was used. Overlay 1 had a final composition of DMEM, 0.8% Noble agar, 1%BCS, 0.2% NaHCO3, 50mM MgCl2, 1% non-essential amino acids. Overlay 2 had a final composition of DMEM, 0.1%BSA, 40mM MgCl2, 0.2% glucose, 2mM sodium pyruvate, 4mM L-glutamine, 4mM oxaloacetic acid (Sigma), and 0.2% NaHCO3. Plaque assays were incubated for 72 hrs at 37oC. Cells were then fixed with 10%TCA (Sigma) and stained with 0.1% crystal violet (Sigma) in 20% ethanol.

Example 1: Experimental Results

[00148] Genomic analysis of the 42 Tem 8+ cell lines refractory to SVV infection revealed robust expression of RNAs encoding for components of the innate immune response specifically the ISG product IFI35 (data not shown). These data suggest that the type I IFN pathway modulates tropism of SVV infection (24). This signaling pathway is known to define tropism of picornaviruses related to SVV such as poliovirus, enterovirus A71 and human rhinovirus (HRV) (60-63). Removal of the type I IFN receptor or toll-like receptor (TLR) 3 allows for poliovirus replication in tissues not known be normally infected (60, 61), and a single nucleotide polymorphism within the Mda-5 gene results in a protein that is unable to bind to its double stranded RNA ligand, failing to induce the innate immune response, and impairing efficient resolution of enterovirus and HRV infections (62, 63).

[00149] To determine if the innate immune response can restrict SVV replication, Per.C6 cells, known to be susceptible and permissive to SVV infection, were infected with SVV in the presence or absence of IFN-α. Per.C6 are human embryonic retinal cells transformed with the E1 region of adenovirus type 5 and used for SVV growth and titration. Supernatants were harvested 24 after infection and virus titers were determined by plaque assay (Fig. 1). The presence of IFN-α in the medium significantly reduced production of infectious SVV (Fig. 1), suggesting that this cytokine impair SVV replication.

[00150] To determine if susceptible but non-permissive cells are unable to support SVV infection due to a type I IFN response, Tem 8+ HeLa cells were infected with SVV at an MOI of 1 in the presence of the JAK/STAT signaling inhibitor staurosporine (SPP). This treatment increased production of infectious SVV, showing that SSP can convert non-permissive to permissive cells (Fig. 2A). Pre-treatment of permissive Per.C6 cells with IFN-α reduced virus yields. However, the presence of SSP reversed the inhibitory effect of IFN-α on SVV replication (Fig. 2B).

[00151] Removal of acetyl moieties from histones is catalyzed by enzymes known as histone deacetylases (HDACs) and leads to DNA transcription. Chemical inhibition of these proteins (HDACi) retards RNA synthesis, resulting in an impairment of cell growth and proliferation. Consequently, several HDACi have been approved by the Food and Drug Administration (FDA) for the treatment of cutaneous/peripheral T-cell lymphomas, hematological malignancies and solid tumors (reviewed in (64)). HDACi can also specifically dampen the type I IFN response (65-71). To determine if HDAC inhibition can confer

permissivity to HeLa cells that are minimally permissive to SVV infection, cells were grown in the presence of 2mM Trichostatin A for 24 hours and then infected with SVV. Sixteen hours after infection virus yields were determined by plaque assay (Fig. 3A). By inhibiting HDAC, SVV infection of non-permissive HeLa cells was observed to be more efficient, thereby expanding cell tropism of the virus. Pre-treatment of permissive Per.C6 cells with IFN-α reduced virus yields. However, the presence of Trichostatin A reversed the inhibitory effect of IFN-α on SVV replication (Fig. 3B).

[00152] Synthesis of IFN is regulated in a PI3K-AKT-mTOR dependent fashion via translation of mRNAs encoding the transcription factors IRF5 and IRF7 (72, 73). Inhibition of the mTORC1 complex has been shown to reduce type I IFN production. Torin 2, a specific inhibitor of mTORC1, was tested for promoting SVV replication. Treatment of semi-permissive HeLa cells with Torin 2 prior to infection substantially increased SVV yields (Fig. 4A). This enhanced replication by Torin 2 was not reduced in the presence of IFN-α. Furthermore, Torin 2 promoted SVV replication in permissive Per.C6 cells even in the presence of IFN-a (Fig. 4B).

[00153] Overall these experiments demonstrated that treatment of susceptible but minimally permissive HeLa cells with staurosporine, a known inhibitor of JAK/STAT signaling, Trichostatin A, an inhibitor of histone deacetylases, or Torin 2, a second generator inhibitor of the mTORC1 complex promoted efficient SVV infection. In contrast, pre-treatment of susceptible and permissive Per.C6 cells with type I interferon (IFN) alone significantly reduced virus replication, while addition of any of the three compounds to cell pretreated with IFN restored SVV replication.

Example 2: Discussion

[00154] Immunotherapies, including oncolytic virus therapies alone or in combination with therapies known to modulate the tumor microenvironment, are revolutionizing the treatment of cancer, making treatments possible for incurable, hard to target and/or aggressive cancers. The antitumor activity of oncolytic viruses results from virus associated immunogenic cell death and induction of an immune response against tumor specific antigens. The majority of oncolytic viruses being used in the clinic including T-VEC (herpes simplex virus type I engineered to synthesis GMCSF) and PVSRIPO (P1/Sabin modified with the 5'-untranslated region of human rhinovirus type 2), are directed to cells of the tumor proper, as are the monoclonal antibodies used in antibody-based immunotherapies. The rapid rise of resistance by the tumor to these

treatments can reduce the utility of these therapies. Targeting multiple components of the tumor microenvironment such as the surrounding stromal cells, which can comprise 90% of the tumor mass, the angiogenic epithelial cells and tumor associated fibroblasts, as well as the malignant cells themselves should enhance the efficacy of the therapeutic. The presence of a common factor, such as Tem 8 on the surface of the various cellular constituents of this microenvironment suggests that the generation of one therapeutic agent may be feasible and the most promising.

[00155] The results reported here suggest that dampening the type I IFN response in three different ways allows SVV replication in otherwise poorly permissive HeLa cells. Inhibition of the production of type I IFN by Torin 2, down-regulation of the type I IFN receptor on the cell surface, and blocking STAT1 signaling would all be expected to reduce the synthesis of the antiviral IFN-induced proteins. All three treatments led to increased replication of SVV in HeLa cells. Furthermore, these inhibitors rendered SVV replication in Per.C6 cells, which are fully permissive for viral replication, resistant to the inhibitory effects of IFN-α. Whether SVV replication in other semi-permissive tumor cell lines would be enhanced by these treatments is the object of current study.

[00156] It is known that treatment of engrafted human tumors in mice with other oncolytic viruses, such as vesicular stomatitis virus or herpes simplex virus type 1 in the presence of the first-generation mTOR inhibitor rapamycin or HDAC inhibitors lead to tumor regression. It was hypothesized whether treatment of human tumors in mice with SVV plus one or more of the inhibitors of the IFN pathway reported here would lead to similar findings. The benefit of an SVV based treatment for cancers is the pinpoint specificity of viral infection due to the limited expression of the viral receptor Tem8 on tumor cells or cells within the immediate tumor microenvironment, including the vascular and stromal cells. The combination of viral and pharmacologic (i.e. inhibitors of the IFN pathway) should allow for efficient SVV replication within the tumor, leading to cell lysis and stimulation of anti-tumor cell immunity.

[00157] SVV can be particularly successful and safe in patients thanks to ANTXR1/TEM8 capacity of being able to be expressed on the surface of tumor cells, while other viruses do not have such specific viral receptor and IFN I inhibitor would be harmful for the patients.

[00158] Accordingly, these results support the inventions as described in the Summary Section above, and claimed below.

[00159] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations, and subcombinations of ranges for specific embodiments therein are intended to be included.

[00160] The disclosures of each patent, patent application, and publication cited or described in this document are hereby incorporated herein by reference, in its entirety.

[00161] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

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CLAIMS

What is claimed:

1. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an interferon type I (IFN-I) inhibiting agent comprising an mTOR inhibitor and an effective amount of Seneca Valley Virus (SVV) or SVV derivative, wherein the cancer is characterized by:
    a. an expression level of anthrax toxin receptor 1 (ANTXRl) higher than an ANTXRl reference value, and
    b. an expression level of IFN-I higher than an IFN-I reference value.

2. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor and an effective amount of SVV or SVV derivative, wherein the cancer is characterized by an expression level of ANTXRl higher than an ANTXRl reference value, and wherein the IFN-I inhibiting agent reduces the expression level of IFN-I in the cancer thereby favoring replication of the SVV or the SVV derivative and reducing or eliminating the cancer.

3. A method of predicting the efficacy of a Seneca Valley Virus (SVV) treatment, or an SVV derivative treatment of a cancer in a subject in need thereof, the method comprising determining the expression level of ANTXRl and the expression level of IFN-I in the cancer from the subject, wherein:
    a. an expression level of ANTXRl higher than an ANTXRl reference value, and
    b. an expression level of IFN-I higher than an IFN-I reference value

    are predictive that the treatment is effective, and wherein when the treatment is predicted to be effective, recommending treatment of the subject; and
    wherein the treatment comprises administering to the subject an IFN-I inhibiting agent comprising an mTOR inhibitor.

4. A pharmaceutical composition for treating a cancer in a subject in need thereof, the pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative and a pharmaceutical acceptable carrier.

5. The use of a pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative for treating a cancer in a patient in need thereof.

6. The use of a pharmaceutical composition comprising an IFN-I inhibiting agent comprising an mTOR inhibitor, an SVV or an SVV derivative in the manufacture of a drug for treating a cancer in a patient in need thereof.

7. The method of any one of claims 1-3, wherein the expression level of ANTXR1 is determined based on the level of an ANTXR1 mRNA or an ANTXR1 protein.

8. The method of any one of claims 1-3, wherein the expression level of IFN-I is determined based on the level of an IFN-I biomarker mRNA or an IFN-I biomarker protein.

9. The method or pharmaceutical composition of any one of claims 1-8, wherein the subject is administered at least one anti-cancer therapeutic agent selected from the group consisting of: a checkpoint inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a cytokine, a growth factor, a photosensitizing agent, a toxin, a siRNA molecule, a signaling modulator, an anti-cancer antibiotic, an anti-cancer antibody, an angiogenesis inhibitor, a chemotherapeutic compound, anti-metastatic compound, an immunotherapeutic compound, a CAR therapy, a dendritic cell-based therapy, a cancer vaccine, an oncolytic virus, an engineered anti-cancer virus or virus derivative and a combination of any thereof.

10. The method or pharmaceutical composition of claim 9, wherein the at least one anti-cancer therapeutic agent is administered formerly, simultaneously or subsequently to the administering of the SVV.

11. The method or pharmaceutical composition of any one of claims 1-10, wherein the mTOR inhibitor inhibits at least one of mTORC1 and mTORC2.

12. The method or pharmaceutical composition of claim 11, wherein the mTOR inhibitor is Torin 2.

13. The method or pharmaceutical composition of any one of claims 1-12, wherein the cancer comprises a triple negative breast cancer, small cell lung cancer, a non-small squamous cell carcinoma, a skin cancer, a hepatocellular carcinoma, a colon cancer, a cervical cancer, an ovarian cancer, an endometrial cancer, a pancreatic cancer, a thyroid cancer, a kidney cancer, a bone cancer, an oesophagus cancer, a soft tissue cancer or any cancer expressing ANTXR1.

14. The method or pharmaceutical composition of claim 13, wherein the cancer comprises a cervical cancer.

15. The method or pharmaceutical composition of any one of claims 1-14, wherein the subject is further administered at least one additional IFN-I inhibiting agent selected from the group consisting of: HDAC inhibitor, JAK/STAT inhibitor, IFN inhibitor, IFN antibody, IFN-α Receptor 1 antibody, IFN-α Receptor 2 antibody and viral peptide and a combination of any thereof.

16. The method of claim 16, wherein the HDAC inhibitor is Trichostatin A.

17. The method of any one of claims 15-16, wherein the JAK/STAT inhibitor is staurosporine.

18. A Seneca Valley Virus (SVV) or SVV derivative in combination with IFN-I inhibiting agent comprising an mTOR inhibitor for use in the manufacture of a medicament for treatment of a cancer, wherein the cancer is characterized by an expression level of anthrax toxin receptor 1 (ANTXRl) higher than an ANTXRl reference value, and wherein the IFN-I inhibiting agent reduces the expression level of IFN-I in the cancer thereby

favoring replication of the SVV or the SVV derivative and reducing or eliminating the cancer.

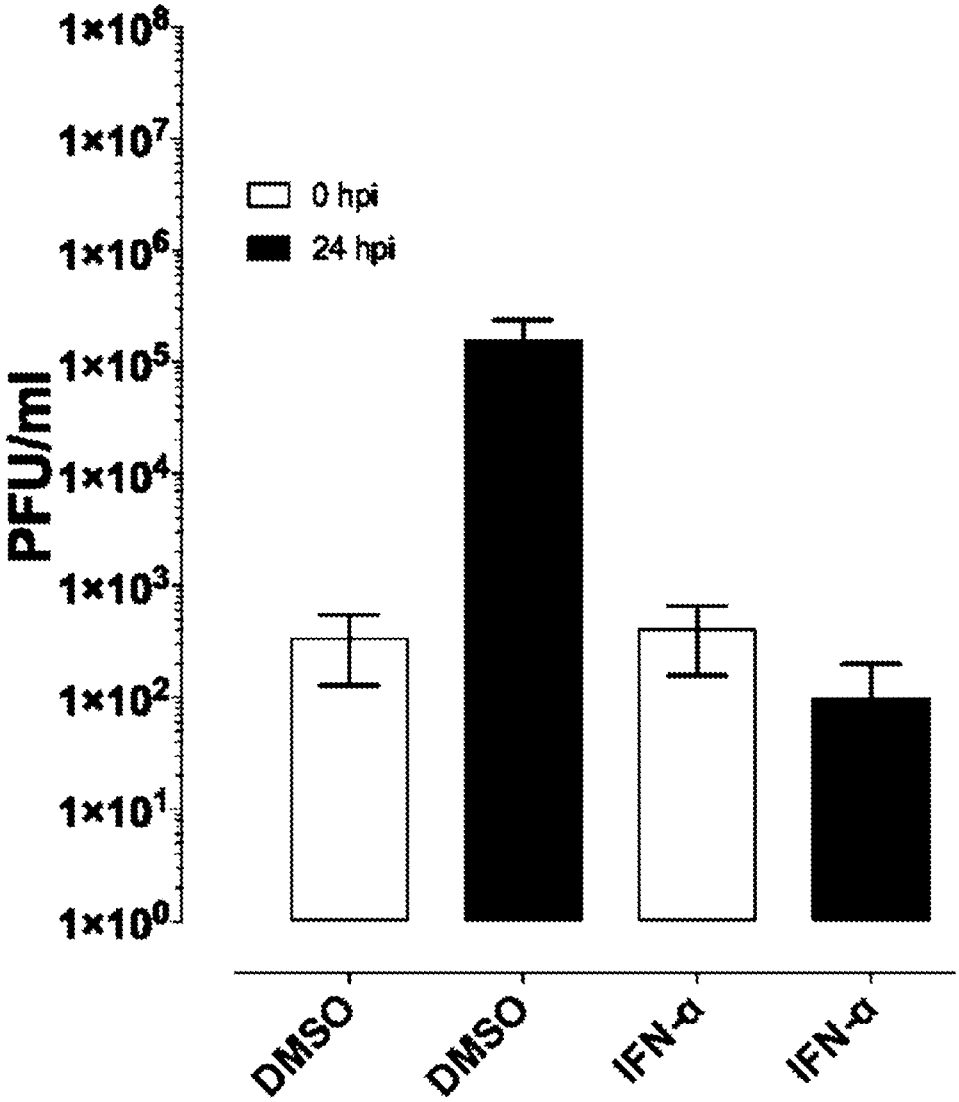

1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
PFU/ml
0 hpi
24 hpi
DMSO
DMSO
IFN-α
IFN-α

FIG. 1

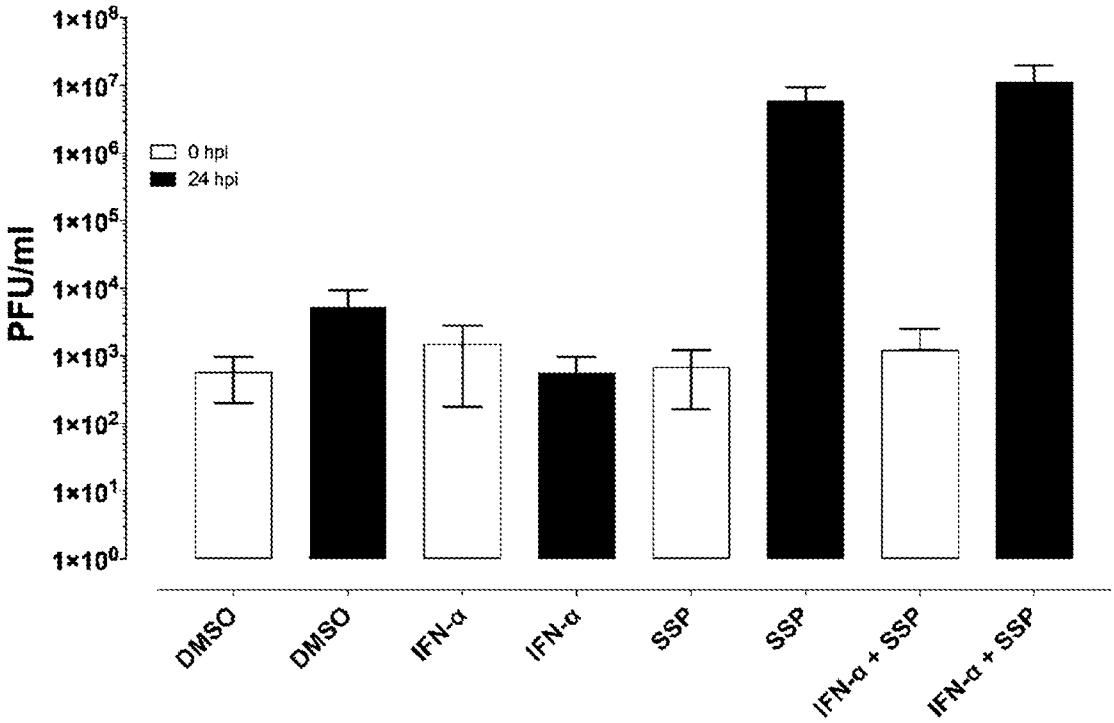

PFU/ml
1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
0 hpi
24 hpi
DMSO
DMSO
IFN-α
IFN-α
SSP
SSP
IFN-α + SSP
IFN-α + SSP

FIG. 2A

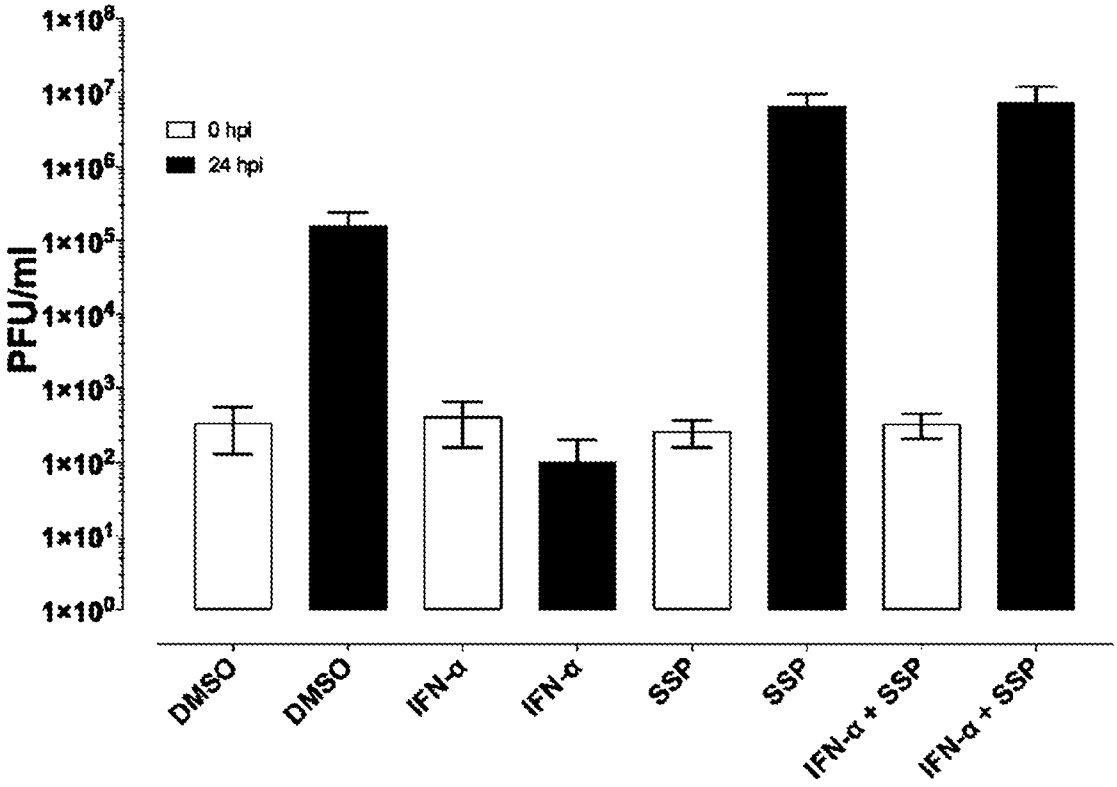

1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
PFU/ml
0 hpi
24 hpi
DMSO
DMSO
IFN-α
IFN-α
SSP
SSP
IFN-α + SSP
IFN-α + SSP

FIG. 2B

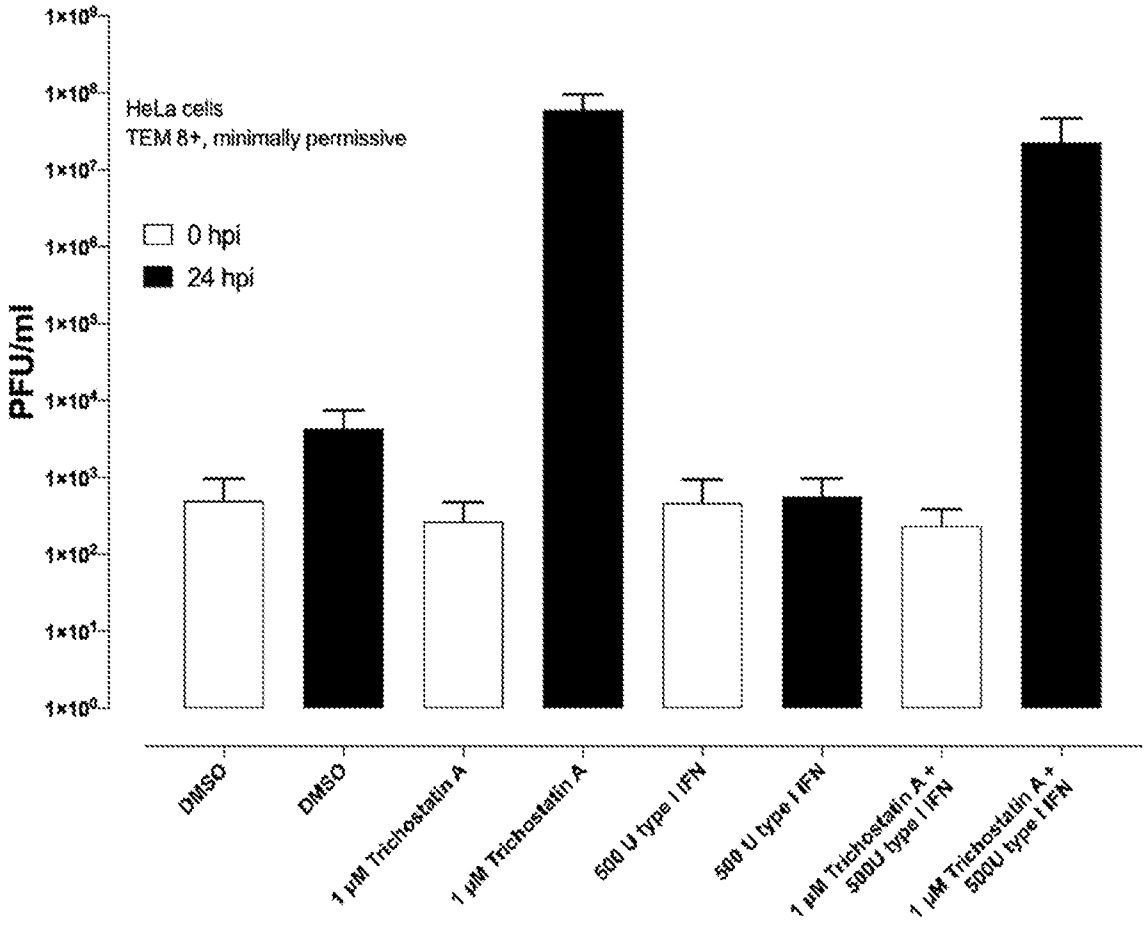

HeLa cells
TEM 8+, minimally permissive
0 hpi
24 hpi
PFU/ml
1×10⁸
1×10⁸
1×10⁷
1×10⁶
1×10⁵
1×10⁴
1×10³
1×10²
1×10¹
1×10⁰
DMSO
DMSO
1 μM Trichostatin A
1 μM Trichostatin A
500 U type I IFN
500 U type I IFN
1 μM Trichostatin A + 500U type I IFN
1 μM Trichostatin A + 500U type I IFN

FIG. 3A

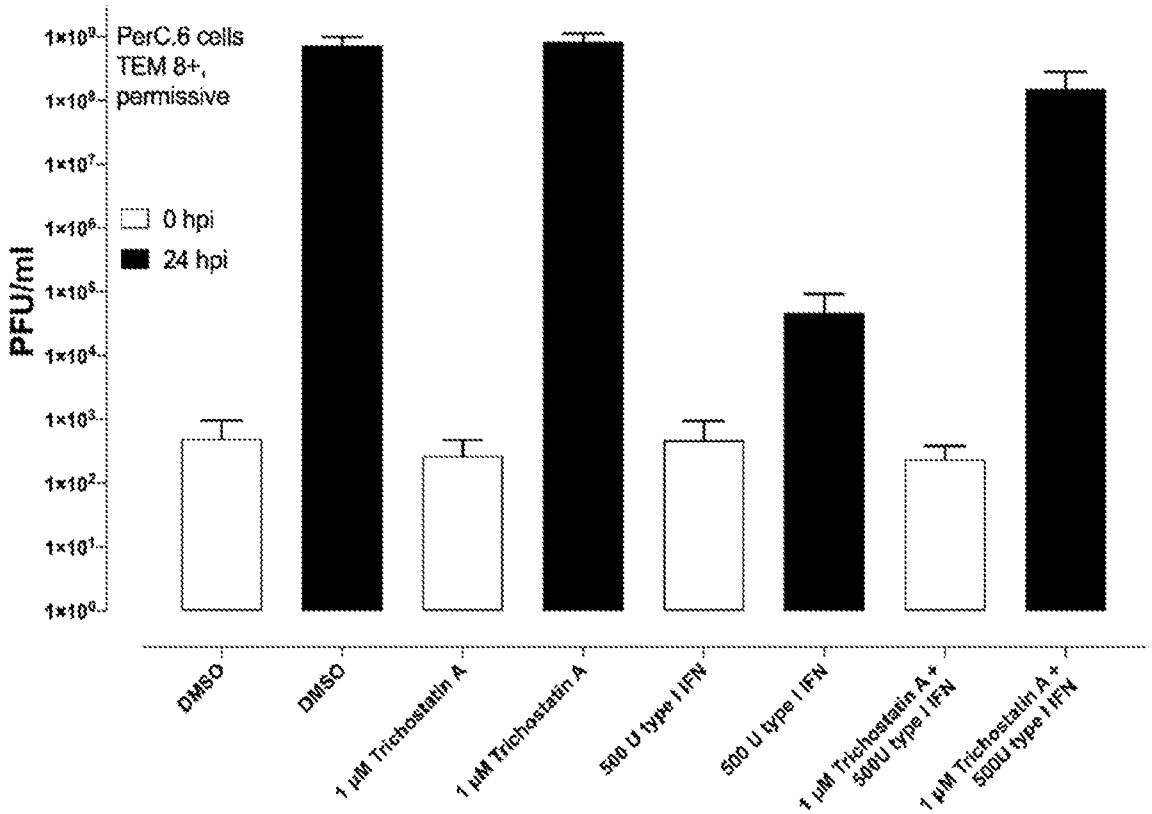

PerC.6 cells
TEM 8+,
permissive
0 hpi
24 hpi
PFU/ml
1×10⁹
1×10⁸
1×10⁷
1×10⁶
1×10⁵
1×10⁴
1×10³
1×10²
1×10¹
1×10⁰
DMSO
DMSO
1 µM Trichostatin A
1 µM Trichostatin A
500 U type I IFN
500 U type I IFN
1 µM Trichostatin A + 500U type I IFN
1 µM Trichostatin A + 500U type I IFN

FIG. 3B

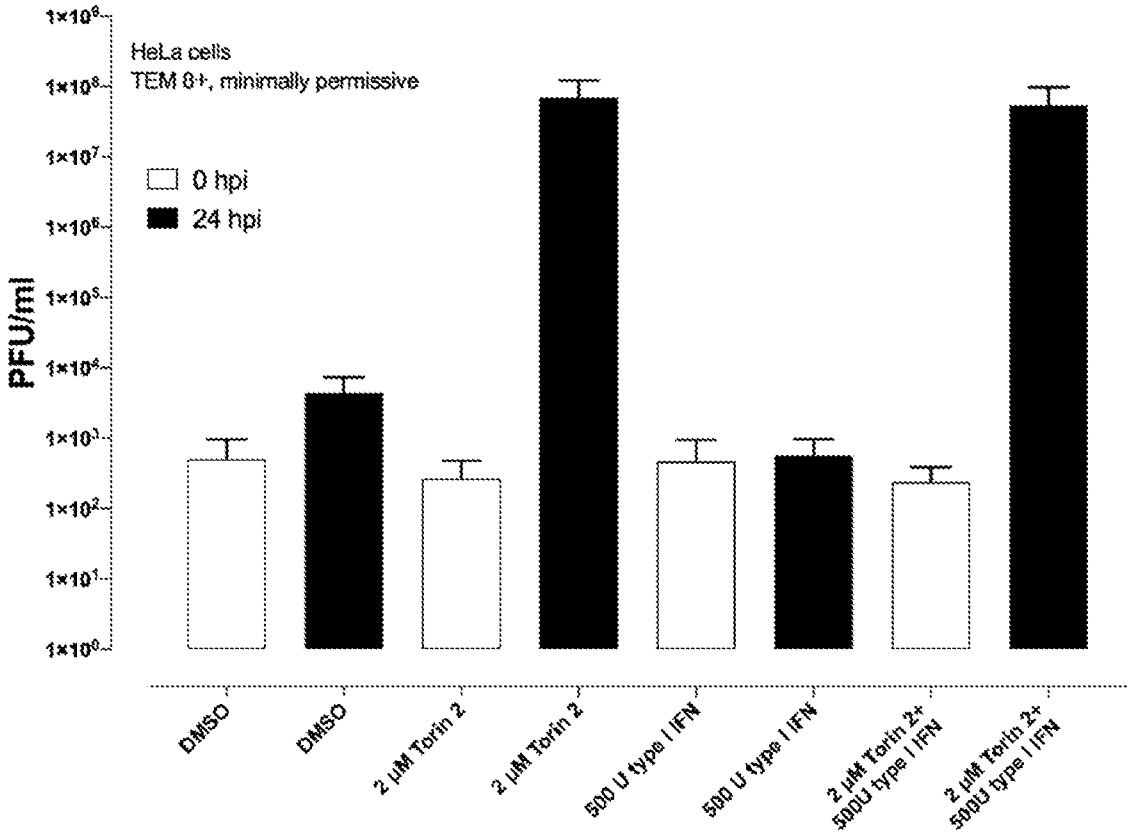

HeLa cells
TEM 8+, minimally permissive
0 hpi
24 hpi
PFU/ml
1×10⁹
1×10⁸
1×10⁷
1×10⁶
1×10⁵
1×10⁴
1×10³
1×10²
1×10¹
1×10⁰
DMSO
DMSO
2 μM Torin 2
2 μM Torin 2
500 U type I IFN
500 U type I IFN
2 μM Torin 2+ 500U type I IFN
2 μM Torin 2+ 500U type I IFN

FIG. 4A

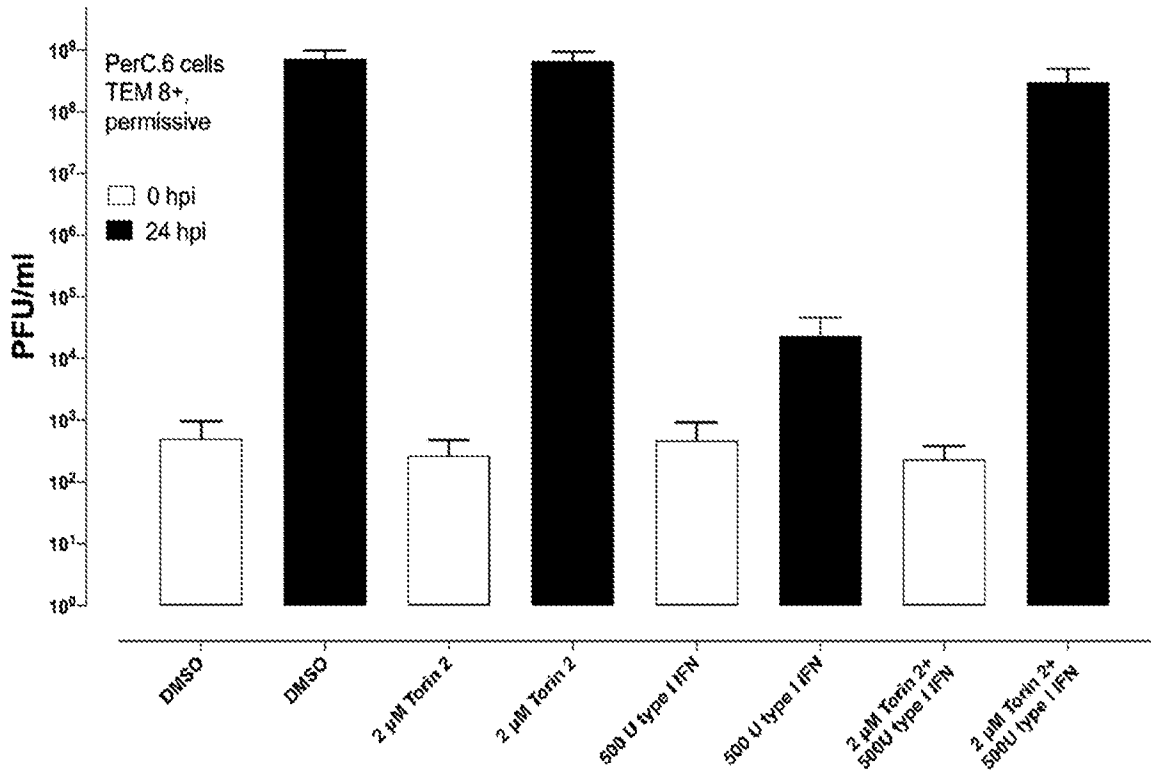

PerC.6 cells
TEM 8+,
permissive
0 hpi
24 hpi
PFU/ml
DMSO
DMSO
2 µM Torin 2
2 µM Torin 2
500 U type I IFN
500 U type I IFN
2 µM Torin 2+
500U type I IFN
2 µM Torin 2+
500U type I IFN

FIG. 4B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/42795

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/768, A61P 35/00, C12N 1/36, C12N 15/74 (2020.01)

CPC - A61K 35/768, A61P 35/00, C12N 1/36, C12N 15/74, G01N 33/57484, G12N 2800/52

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.
Y	WO 2017/096201 Al (MEMORIAL SLOAN-KETTERING CANCER CENTER) 8 June 2017 (08.06.2017). Especially para [0004], para [0005], para [0008], para [0027], para [0028], para [0044], para [0045], para [0047], para [0052], para [0086], para [0090] and para [0091].	1-8 and 18
Y	CHANDRA et al. HCV Infection Selectively Impairs Type I but Not Type III IFN Signaling. The American Journal of Pathology, January 2014, Vol 184, No 1, pp214-229. Especially pg 224, col 1, para 3.	1-8 and 18

☐ 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
"D" document cited by the applicant in the international application
"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
02 October 2020	28 OCT 2020
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 Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/42795

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.: 9-17
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:

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.:

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.