ABROGATING PROINFLAMMATORY CYTOKINE PRODUCTION DURING ONCOLYTIC REOVIRUS THERAPY

BACKGROUND

Reovirus is a dsRNA virus with tropism to cancer cells having an activated Ras pathway. It has been demonstrated that administration of reovirus into tumor bearing animals results in generation of a robust anti-viral response mediated by both the humoral and cellular arms of the immune system. This anti-viral response can antagonize the oncolytic effectiveness of the therapeutic virus. As such, combinational use of immune suppressing agents to overcome this immune antagonism of reovirus oncolysis has been explored. It has been demonstrated that co-administration of agents that ablate the generation of neutralizing anti-reovirus antibodies (NARA) can result in morbidity in the test animals. The response in the test animals has been characterized by reovirus replication outside of the target tumor tissues, suggesting that humoral immunity serves a protective role in preventing reovirus infection of the host (Qiao et al., *Clin. Cancer Res.* 14(1):259-69 (2008)).

SUMMARY

Provided herein are methods for treating a proliferative disorder in a subject comprising administering to the subject one or more reoviruses and one or more agents that modulate expression or activity of pro-inflammatory cytokines. For example, the agents may inhibit expression or activity of pro-inflammatory cytokines.

In one aspect, there is provided the use of one or more reoviruses and

one or more platinum compounds that inhibit expression or activity of a pro-inflammatory cytokine but do not inhibit production of neutralizing anti-reovirus antibodies (NARA) in the manufacture of a medicament for treating a proliferative disorder in a subject.

The details of one or more embodiments are set forth in the accompanying drawings and description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C and 1D show reduced tumor growth and increased survival following reovirus/cisplatin combination therapy. C57Bl/6 (Figures 1A and 1C) and C3H (Figures 1B and 1D) mice bearing subcutaneous B16.F10 and K1735 tumors, respectively, were treated on days 1 and 4 with either reovirus alone via intratumoral (i.t.) injection (squares), cisplatin alone via intraperitoneal (i.p.) administration (triangles), or reovirus and cisplatin in combination (circles). Control treated mice (diamonds) received PBS. Tumors were measured on the days indicated and tumor volume expressed as tumor volume relative to volume at commencement of treatment (Figures 1A and 1B). Mice

were euthanized when tumors exceeded 15mm in any one dimension. Survival is expressed as Kaplan-Myer plots (Figures 1C and 1D).

Figure 2 is a graph showing the neutralizing anti-reovirus antibody (NARA) response after no treatment (control) or treatment with reovirus, cisplatin or the combination of reovirus and cisplatin.

Figures 3A, 3B, 3C, 3D, 3E, 3F, and 3G are graphs showing pro-inflammatory cytokine response is abrogated by cisplatin. The response of IL-1I (Figure 3A), IL-3 (Figure 3B), IL-6 (Figure 3C), IL-12 (Figure 3D), IL-17 (Figure 3E), MIP-1I (Figure 3F) and RANTES (Figure 3G) were measured after no treatment (control) or treatment with reovirus, cisplatin or the combination of reovirus and cisplatin.

DETAILED DESCRIPTION

As described previously (see, for example, U.S. Patent Nos. 6,110,461; 6,136,307; 6,261,555; 6,344,195; 6,576,234; and 6,811,775), reoviruses use a host cell's Ras pathway machinery to downregulate double-stranded RNA-activated protein kinase (PKR) and thus replication in the cell. Based upon these discoveries, methods have been developed for using reoviruses to treat proliferative disorders. It has been demonstrated that reovirus therapy results in release of pro-inflammatory cytokines. The pro-inflammatory cytokines antagonize reovirus infection and reovirus spread into the tumor tissue. The protective function of the humoral arm of the immune system in preventing reovirus toxicity has been further suggested by the observation that while, athymic mice manifest no morbidity to reovirus infection, SCID mice and B-cell knock-out animals invariably die from reovirus infection.

Reovirus oncolysis can be enhanced *in vitro* by the use of cytotoxic agents. Surprisingly, as described herein, the combinational use of platinum compounds does not impact the production of NARA but has a profound effect on the production of pro-inflammatory cytokines including: IL-lI, IL-3, IL-6, IL-12 p70, IL-17, MIP-lI, and RANTES. Inhibition of pro-inflammatory cytokines by cisplatin prevents T-cell recognition of reovirus infected cells and allows virus replication to ensue without cellular immunity antagonism. However, cisplatin allows production of protective neutralizing anti-reovirus antibodies (NARA) NARA and its concomitant benefits (e.g., preventing reovirus toxicities in patients). The use of platinum compounds to selectively block both innate and adaptive T-cell responses, while having no effect on B-cell activity, has not previously been described.

Provided herein is a method of treating a proliferative disorder in a subject comprising administering a reovirus to the subject and administering to the subject an agent that modulates pro-inflammatory cytokines.

For example, the agent inhibits the expression or activity of the pro-inflammatory cytokines. As used herein, the term modulate refers to a change (positive or negative) of 10, 20, 30, 40, 50, 60, 70, 80, 90 percent or greater as compared to a control level. As used herein, control refers to a reference standard from an untreated sample or subject. By way of example, a control level is the level of expression or activity in a control sample in the absence of a stimulus. The control can be prior to, after recovery, or without the stimulus.

Optionally, the cytokine modulating agent blocks T-cell responses while having little to no effect on B-cell activity. Thus, the agent inhibits pro-inflammatory cytokines but does not inhibit or minimally inhibits production of NARA. Optionally, the agent is a platinum compound. Suitable platinum compounds include, but are not limited to, cisplatin, carboplatin, metaplatin and oxaliplatin.

Other agents that inhibit pro-inflammatory cytokines include, but are not limited to, TNF-I antibodies such as infliximab, CDP571, CDP870, and adalimumab; recombinant, human soluble p55 TNF receptors such as onercept; soluble TNF receptor and Fc fragment fusion proteins such as etanercept; pegylated Fab fragments of humanized antibody to TNF such as certolizumab pegol; chimeric antibodies to anti-I chain of IL-2 receptor such as basiliximab or daclizumab; IL-12p40 antibodies such as ABT-874; IL-6 receptor antibodies such as MRA or tocilizumab; IFN-K antibodies such as fontolizumab; antibodies that inhibit IL-1 binding to the IL-1 receptor such as AMG108; caspase-1 inhibitors that inhibit cytokine-release such as diarylsulphonylurene; IL-15 antibodies such as mepolizumab; IL-8 antibodies such as ABX-IL-8; IL-9 antibodies including IL-9 monoclonal antibodies; recombinant human IL-21 also referred to as 494C10; inhibitors of TNF-I, IL-19, IL-6 and granulocyte monocyte-colony stimulating factor expression such as biophylum sensitivum; NF-PB signaling blockers that inhibit pro-inflammatory cytokine expression such as simvastatin; and inhibitors of IL-6 expression and NF-PB activation such as (-)-epigallocatechin-3-gallate (EGCG).

Other agents include human recombinant lactoferrin, which inhibits cellular release of proinflammatory cytokines and prometastatic cytokines (including IL-6, IL-8, granulocyte macrophage colony-stimulating factor and TNF-α). Inhibitors of dendritic cell derived IL-12 and IL-18 such as rapamycin and sanglifehrin are also suitable for use

in the provided methods. Rapamycin is an immunosuppressant that inhibits T cell mTOR kinase activation, and Sanglifehrin A is a cyclophilin-binding immunosuppressant that also inhibits IL-2 dependent T cell proliferation. Also suitable for use in the provided methods is dietary rutin colitis, which suppresses the induction of pro-inflammatory cytokines such as IL-1β, IL-6, and GM-CS.

Optionally, the method further includes selecting a subject with a proliferative disorder, wherein the subject is in need of inhibition of a pro-inflammatory cytokine response. For example, such a subject may include a subject with little response to reovirus alone or with a progressive resistance to reovirus therapy.

As used herein, the term proliferative disorder refers to any cellular disorder in which the cells proliferate more rapidly than normal tissue growth. A proliferative disorder includes, but is not limited to, neoplasms, which are also referred to as tumors. A neoplasm can include, but is not limited to, pancreatic cancer, breast cancer, brain cancer (e.g., glioblastoma), lung cancer, prostate cancer, colorectal cancer, thyroid cancer, renal cancer, adrenal cancer, liver cancer, neurofibromatosis 1, and leukemia. A neoplasm can be a solid neoplasm (e.g., sarcoma or carcinoma) or a cancerous growth affecting the hematopoietic system (e.g., lymphoma or leukemia). Other proliferative disorders include, but are not limited to, neurofibromatosis.

Generally, in proliferating disorders for which reovirus is used as a treatment, at least some of the proliferating cells associated with the disorder may have a mutation in which the Ras gene (or an element of the Ras signaling pathway) is activated, either directly (e.g., by an activating mutation in Ras) or indirectly (e.g., by activation of an upstream or downstream element in the Ras pathway). Activation of an upstream element in the Ras pathway includes, for example, transformation with epidermal growth factor receptor (EGFR) or Sos. See, for example, Wiessmuller and Wittinghofer, 1994, *Cellular Signaling* 6(3):247-267; and Barbacid, 1987, *Ann. Rev. Biochem.* 56, 779-827. Activation of a downstream element in the Ras pathway includes, for example, mutation within B-Raf. See, for example, Brose et al., 2002, *Cancer Res.* 62:6997-7000. A proliferative disorder that results, at least in part, by the activation of ras, an upstream element of ras, or an element in the ras signaling pathway is referred to herein as a ras-mediated proliferative disorder. In addition, the reovirus is useful for treating proliferative disorders caused by mutations or dysregulation of PKR. See, for example, Strong et al., 1998, *EMBO J.* 17:3351-62.

Optionally, the provided methods further comprise the step of selecting a subject with a ras-mediated proliferative disorder. Optionally, the provided methods comprise the

step of determining whether the proliferative disorder is a ras-mediated proliferative disorder. Such methods for determining whether a proliferative disorder has a certain phenotype are known. See, for example, U.S. Patent No. 7,306,902, which is incorporated herein by reference in its entirety.

As used herein, reovirus refers to any virus classified in the reovirus genus, whether naturally occurring, modified, or recombinant. Reoviruses are viruses with a double-stranded, segmented RNA genome. The virions measure 60-80 nm in diameter and possess two concentric capsid shells, each of which is icosahedral. The genome consists of double-stranded RNA in 10-12 discrete segments with a total genome size of 16-27 kbp. The individual RNA segments vary in size. Three distinct but related types of reoviruses have been recovered from many species. All three types share a common complement-fixing antigen.

The human reovirus includes three serotypes: type 1 (strain Lang or T1L), type 2 (strain Jones, T2J), and type 3 (strain Dearing or strain Abney, T3D). The three serotypes are easily identifiable on the basis of neutralization and hemagglutinin-inhibition assays. A reovirus according to this disclosure can be a type 3 mammalian orthoreovirus. Type 3 mammalian orthoreoviruses include, without limitation, Dearing and Abney strains (T3D or T3A, respectively). See, for example, ATCC Accession Nos. VR-232 and VR-824.

The reovirus may be naturally occurring or modified. The reovirus is naturally-occurring when it can be isolated from a source in nature and has not been intentionally modified by humans in the laboratory. For example, the reovirus can be from a field source, that is, from a human who has been infected with the reovirus. The reovirus may also be selected or mutagenized for enhanced oncolytic activity.

The reovirus may be modified but still capable of lytically infecting a mammalian cell having an active ras pathway. The reovirus may be chemically or biochemically pretreated (e.g., by treatment with a protease, such as chymotrypsin or trypsin) prior to administration to the proliferating cells. Pretreatment with a protease removes the outer coat or capsid of the virus and may increase the infectivity of the virus. The reovirus may be coated in a liposome or micelle (Chandran and Nibert, J. of Virology 72(1):467-75 1998). For example, the virion may be treated with chymotrypsin in the presence of micelle-forming concentrations of alkyl sulfate detergents to generate a new infectious subviral particle (ISVP).

The reovirus may be a recombinant reovirus. For example, the recombinant reovirus can be a reassortant reovirus, which includes genomic segments from two or more genetically distinct reoviruses. Reassortment of reovirus genomic segments may

occur following infection of a host organism with at least two genetically distinct reoviruses. Reassortment of viruses can be generated in cell culture, for example, by co-infection of permissive host cells with genetically distinct reoviruses. Accordingly, the provided methods include the use of a recombinant reovirus resulting from reassortment of genome segments from two or more genetically distinct reoviruses, including but not limited to, human reovirus, such as type 1 (e.g., strain Lang), type 2 (e.g., strain Jones), and type 3 (e.g., strain Dearing or strain Abney); non-human mammalian reoviruses; or avian reovirus. Recombinant reovirus can also be made by genetic engineering, chemically synthesized, or treatment with chemical or physical mutagens. Optionally, the provided methods include the use of recombinant reoviruses resulting from reassortment of genome segments from two or more genetically distinct reoviruses wherein at least one parental virus is genetically engineered, comprises one or more chemically synthesized genomic segment, has been treated with chemical or physical mutagens, or is itself the result of a recombination event. Optionally, the provided methods include the use of the recombinant reovirus that has undergone recombination in the presence of chemical mutagens, including but not limited to, dimethyl sulfate and ethidium bromide, or physical mutagens, including but not limited to, ultraviolet light and other forms of radiation.

Optionally, the provided methods include the use of reoviruses with mutations (including insertions, substitutions, deletions or duplications) in one or more genome segments. Such mutations can comprise additional genetic information as a result of recombination with a host cell genome or can comprise synthetic genes. For example, mutant reoviruses as described herein can contain a mutation that reduces or essentially eliminates expression of a sigma3 polypeptide or that results in the absence of a functional sigma3 polypeptide as described in U.S. Serial No. 12/124,522, which is incorporated by reference herein in its entirety. A mutation that eliminates expression of a sigma3 polypeptide or that results in the absence of a functional sigma3 polypeptide can be in the nucleic acid encoding the sigma3 polypeptide (i.e., the S4 gene) or in a nucleic acid that encodes a polypeptide that regulates the expression or function of the sigma3 polypeptide.

As used herein, a mutation that reduces the expression of a sigma3 polypeptide refers to a mutation that results in a decrease in the amount of sigma3 polypeptides, compared to a reovirus expressing wild type levels of sigma3 polypeptide, of at least 30% (e.g., at least 40%, 50%, 60%, 70%, 80%, 90%, or 95%). As used herein, a mutation that essentially eliminates expression of a sigma3 polypeptide refers to a mutation that results

in a decrease in the amount of sigma3 polypeptides, relative to the amount of sigma3 polypeptides produced by a wild type reovirus, of at least 95% (e.g., 96%, 97%, 98%, 99%, or 100%). As used herein, a mutation that results in a decrease in or absence of a functional sigma3 polypeptide refers to a mutation that allows expression of the sigma3 polypeptide but that results in a sigma3 polypeptide that is not able to assemble or incorporate into the viral capsid. It would be understood that it may be desirable or necessary for sigma3 polypeptides to retain other functionalities (e.g., the ability to bind RNA) in order that the mutant reovirus retain the ability to propagate.

A mutation in a sigma3 polypeptide as described herein can result in a sigma3 polypeptide that is incorporated into the capsid at levels that are reduced relative to a sigma3 polypeptide that does not contain the mutation (e.g., a wild type sigma3 polypeptide). A mutation in a sigma3 polypeptide as described herein also can result in a sigma3 polypeptide that cannot be incorporated into a viral capsid. Without being bound by any particular mechanism, a sigma3 polypeptide may have reduced function or lack function due, for example, to an inability of the sigma3 polypeptide and the mu1 polypeptide to bind appropriately, or due to a conformational change that reduces or prohibits incorporation of the sigma3 polypeptide into the capsid.

In addition to a mutation that abolishes or reduces expression of the sigma3 polypeptide or that results in a non-functional or reduced-function sigma3 polypeptide, a mutant reovirus as described herein also can contain one or more further mutations (e.g., a second, third, or fourth mutation) in one of the other reovirus capsid polypeptides (e.g., mu1, lambda2, and/or sigma1). Reoviruses containing a mutation affecting the sigma3 polypeptide and, optionally, a further mutation in any or all of the other outer capsid proteins can be screened for the ability of such mutant reoviruses to infect and cause lysis of cells. For example, neoplastic cells that are resistant to lysis by wild type reovirus can be used to screen for effective mutant reoviruses described herein.

For example, a further mutation can reduce or essentially eliminate expression of a mu1 polypeptide or result in the absence of a functional mu1 polypeptide. The mu1 polypeptide, which is encoded by the M2 gene, is likely involved in cell penetration and may play a role in transcriptase activation. Each virion contains about 600 copies of mu1 polypeptides, which are present in the form of 1:1 complexes with sigma3 polypeptides. The mu1 polypeptide is myristolated on its N-terminus, and then the myristolated N-terminal 42 residues are cleaved off, resulting in a C-terminal fragment (mu1C). Additionally or alternatively, a further mutation can reduce or essentially eliminate expression of a lambda2 polypeptide or result in the absence of a functional lambda2

polypeptide, and/or a further mutation can reduce or essentially eliminate expression of a sigma1 polypeptide or result in the absence of a functional sigma1 polypeptide. The lambda2 polypeptide is encoded by the L2 gene and is involved in particle assembly, and exhibits guanylyltransferase and methyltransferase activity. The sigma1 polypeptide is encoded by the S1 gene and is involved in cell-attachment and serves as the viral hemagglutinin.

For example, the reovirus has a lambda-3 polypeptide having one or more amino acid modifications; a sigma-3 polypeptide having one or more amino acid modifications; a mu-1 polypeptide having one or more amino acid modifications; and/or a mu-2 polypeptide having one or more amino acid modifications, as described in U.S. Serial No. 12/046,095, which is incorporated by reference herein in its entirety. By way of example, the one or more amino acid modifications in the lambda-3 polypeptide are a Val at residue 214, an Ala at residue 267, a Thr at residue 557, a Lys at residue 755, a Met at residue 756, a Pro at residue 926, a Pro at residue 963, a Leu at residue 979, an Arg at residue 1045, a Val at residue 1071, or any combination thereof, numbered relative to GenBank Accession No. M24734.1. It is noted that, when the amino acid sequence is a Val at residue 214 or a Val at residue 1071, the amino acid sequence further includes at least one additional change in the amino acid sequence. Optionally, the lambda-3 polypeptide includes the sequence shown in SEQ ID NO:18. Further by way of example, the one or more amino acid modifications in the sigma-3 polypeptide are a Leu at residue 14, a Lys at residue 198, or any combination thereof, numbered relative to GenBank Accession No. K02739. It is noted that, when the amino acid sequence is a Leu at residue 14, the amino acid sequence further includes at least one additional change in the amino acid sequence. Optionally, the sigma-3 polypeptide includes the sequence shown in SEQ ID NO:14. Further by way of example, the one or more amino acid modifications in the mu-1 polypeptide is an Asp at residue 73 numbered relative to GenBank Accession No. M20161.1. Optionally, the mu-1 polypeptide includes the sequence shown in SEQ ID NO:16. Also by way of example, the amino acid modification mu-2 polypeptide is a Ser at residue 528 numbered relative to GenBank Accession No. AF461684.1. Optionally, the mu-1 polypeptide includes the sequence shown in SEQ ID NO:15. A reovirus as described herein having one or more modifications can further include a reovirus sigma-2 polypeptide. Such a sigma-2 polypeptide has a Cys at one or more of position 70, 127, 195, 241, 255, 294, 296, or 340, numbered relative to GenBank Accession No. NP_694684.1. Optionally, the sigma-2 polypeptide includes the sequence shown in SEQ ID NO:12.

Optionally, the reovirus has a L1 genome segment having one or more nucleic acid modifications; a S4 genome segment having one or more nucleic acid modifications; a M1 genome segment having one or more nucleic acid modifications; and/or a M2 genome segment having one or more nucleic acid modifications, as described in U.S. Serial No. 12/046,095, which is incorporated by reference herein in its entirety. By way of example, the one or more nucleic acid modifications in the L1 genome segment are a T at position 660, a G at position 817, an A at position 1687, a G at position 2283, an ATG at positions 2284-2286, a C at position 2794, a C at position 2905, a C at position 2953, an A at position 3153, or a G at position 3231, numbered relative to GenBank Accession No. M24734.1. Optionally, the L1 genome segment includes the sequence shown in SEQ ID NO:8. Further by way of example, the one or more nucleic acid modifications in the S4 genome segment is an A at position 74 and an A at position 624, numbered relative to GenBank Accession No. K02739. Optionally, the S4 genome segment includes the sequence shown in SEQ ID NO:4. Further by way of example, the nucleic acid modification in the M2 genome segment can be a C at position 248, numbered relative to GenBank Accession No. M20161.1. In one embodiment, the M2 genome segment includes the sequence shown in SEQ ID NO:6. Also by way of example, the nucleic acid modification in the M1 genome segment is a T at position 1595, numbered relative to GenBank Accession No. AF461684.1. Optionally, the M1 genome segment includes the sequence shown in SEQ ID NO:5. A reovirus as described herein can include any modification or combination of modifications disclosed herein. Optionally, a reovirus as described herein includes genomic segments having the sequences shown in SEQ ID NOs:1-10 or the polypeptides shown in SEQ ID NOs:11, 12, and 16-21, and either or both SEQ ID NO:13 or 14. Optionally, a reovirus as disclosed herein is identified as IDAC Accession No. 190907-01.

A mutation or modification as referred to herein can be a substitution, insertion or deletion of one or more nucleotides. Point mutations include, for example, single nucleotide transitions (purine to purine or pyrimidine to pyrimidine) or transversions (purine to pyrimidine or *vice versa*) and single- or multiple-nucleotide deletions or insertions. A mutation in a nucleic acid can result in one or more conservative or non-conservative amino acid substitutions in the encoded polypeptide, which may result in conformational changes or loss or partial loss of function, a shift in the reading frame of translation (frame-shift) resulting in an entirely different polypeptide encoded from that point on, a premature stop codon resulting in a truncated polypeptide (truncation), or a mutation in a reovirus nucleic acid may not change the encoded polypeptide at all (silent

or nonsense). See, for example, Johnson and Overington, 1993, *J. Mol. Biol.* 233:716-38; Henikoff and Henikoff, 1992, *Proc. Natl. Acad. Sci. USA* 89:10915-19; and U.S. Patent No. 4,554,101 for disclosure on conservative and non-conservative amino acid substitutions.

Mutations can be generated in the nucleic acid of a reovirus using any number of methods known in the art. For example, site directed mutagenesis can be used to modify a reovirus nucleic acid sequence. One of the most common methods of site-directed mutagenesis is oligonucleotide-directed mutagenesis. In oligonucleotide-directed mutagenesis, an oligonucleotide encoding the desired change(s) in sequence is annealed to one strand of the DNA of interest and serves as a primer for initiation of DNA synthesis. In this manner, the oligonucleotide containing the sequence change is incorporated into the newly synthesized strand. See, for example, Kunkel, 1985, *Proc. Natl. Acad. Sci. USA* 82:488; Kunkel et al., 1987, *Meth. Enzymol.* 154:367; Lewis and Thompson, 1990, *Nucl. Acids Res.* 18:3439; Bohnsack, 1996, *Meth. Mol. Biol.* 57:1; Deng and Nickoloff, 1992, *Anal. Biochem.* 200:81; and Shimada, 1996, *Meth. Mol. Biol.* 57:157. Other methods are used routinely in the art to modify the sequence of a protein or polypeptide. For example, nucleic acids containing a mutation can be generated using PCR or chemical synthesis, or polypeptides having the desired change in amino acid sequence can be chemically synthesized. See, for example, Bang and Kent, 2005, *Proc. Natl. Acad. Sci. USA* 102:5014-9 and references therein.

Nucleic acids from reovirus particles can be isolated using standard commercially available nucleic acid methodology. See also, for example, Schiff et al., "Orthoreoviruses and Their Replication," Ch 52, in *Fields Virology*, Knipe and Howley, eds., 2006, Lippincott Williams and Wilkins. As used herein, isolated nucleic acids refer to nucleic acids that are separated from other nucleic acids with which they are usually associated. Thus, an isolated nucleic acid includes, without limitation, reoviral nucleic acid that is essentially free of non-reoviral (e.g., host cell) nucleic acid, or a reoviral genomic segment that is essentially free of nucleic acid corresponding to other genomic segments. In addition, an isolated nucleic acid can include an engineered nucleic acid such as a recombinant or synthetic nucleic acid.

A mutant reovirus as described herein can be generated by reconstituting genome segments containing at least a mutation or modification using methods known in the art. See, for example, Schiff et al., "Orthoreoviruses and Their Replication," Ch 52, in *Fields Virology*, Knipe and Howley, eds., 2006, Lippincott Williams and Wilkins; Smith et al., 1969, *Virology* 39(4):791-810; and U.S. Patent Nos. 7,186,542; 7,049,127; 6,808,916;

and 6,528,305. A mutant reovirus also can be generated by expressing the reovirus genome segments using a plasmid-based reverse genetic system to produce an ISVP. See, for example, Kobayashi et al., 2007, *Cell Host and Microbe* 1:147-57. As used herein, a genetically-engineered or mutant ISVP is a mutant reovirus and refers to an ISVP generated from a reovirus carrying a genetically-engineered or a spontaneously generated mutation affecting at least the sigma3 polypeptide. The ISVPs described herein are stable and can be propagated as ISVPs for multiple (e.g., more than one, e.g., 2, 3, 4, 5, 10, 20, 50, or more) passages.

The mutant reoviruses described herein, produced via a genetically-engineered ISVP or via a plasmid-based reverse genetic system, can be cultured in, for example, human neoplastic cells or L929 mouse fibroblast cells. Mutant reoviruses disclosed herein can be cultured in cells that are only permissive to reovirus strains lacking the sigma3 polypeptide. Using such cell lines to passage the mutant reoviruses described herein can allow for selection of the mutants and also can be used to reduce or prevent reversions of the mutation(s).

The mutant reoviruses described herein, optionally, exhibit increased infectivity and/or decreased immunogenicity as compared to a non-mutant reovirus (e.g., a control reovirus) and can be selected on the basis of such traits. Increased infectivity can be evidenced by an increase in the range of neoplastic cells and/or the number of cells that are infected by a mutant reovirus compared to a reovirus that expresses a functional sigma3 polypeptide (e.g., an intact virion; e.g., a wild type reovirus). Decreased immunogenicity of mutant reoviruses can be evidenced by the inability of such mutant reoviruses to induce a significant immune response in the subject. The mutant reoviruses described herein also can be screened and selected for other desirable traits including, but not limited to, a faster rate of replication; a faster rate of packaging; the ability to induce apoptosis; the ability to affect lysis in and effectively kill human neoplastic cells lines; the ability to release effective tumor epitopes; interaction with standard chemotherapies; and an increased number of viral progeny. Additionally, mutant reoviruses can be selected for the ability to lytically infect a neoplastic cell (e.g., a mammalian cell having an active Ras pathway). See, for example, U.S. Patent No. 7,052,832.

The reovirus is optionally a reovirus modified to reduce or eliminate an immune reaction to the reovirus. Such a modified reovirus is referred to herein as an immunoprotected reovirus. Such modifications include, but are not limited to, packaging of the reovirus in a liposome, a micelle, or other vehicle to mask the reovirus from the immune system. Alternatively, the outer capsid of the reovirus virion particle may be

removed since the proteins present in the outer capsid are the major determinant of the host humoral and cellular responses.

Reoviruses can be purified using standard methodology. See, for example, Schiff et al., "Orthoreoviruses and Their Replication," Ch 52, in *Fields Virology*, Knipe and Howley, eds., 2006, Lippincott Williams and Wilkins; Smith et al., 1969, *Virology* 39(4):791-810; and U.S. Patent Nos. 7,186,542; 7,049,127; 6,808,916; and 6,528,305. As used herein, purified mutant reoviruses refer to reoviruses that have been separated from cellular components that naturally accompany them. Typically, reoviruses are considered purified when they are at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, or 99%) by dry weight, free from the proteins and other cellular components with which they are naturally associated.

The herein provided reoviruses and agents can be administered *in vitro* or *in vivo* in a pharmaceutically acceptable carrier. Thus, pharmaceutical compositions that include a reovirus and/or agent that inhibits pro-inflammatory cytokines as described herein are provided. See, for example, U.S. Patent No. 6,576,234 regarding reoviruses. In addition to one or more reoviruses and/or agents that inhibit pro-inflammatory cytokines, a pharmaceutical composition typically includes a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier can be a solid, semi-solid, or liquid material that can act as a vehicle, carrier or medium for the reovirus. Thus, compositions containing a reovirus and/or the provided agents can be in the form of tablets, pills, powders, lozenges, sachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

Optionally, the compositions containing a reovirus are suitable for infusion. For intravenous infusions, there are two types of fluids that are commonly used, crystalloids and colloids. Crystalloids are aqueous solutions of mineral salts or other water-soluble molecules. Colloids contain larger insoluble molecules, such as gelatin; blood itself is a colloid. The most commonly used crystalloid fluid is normal saline, a solution of sodium chloride at 0.9% concentration, which is close to the concentration in the blood (isotonic). Ringer's lactate or Ringer's acetate is another isotonic solution often used for large-volume fluid replacement. A solution of 5% dextrose in water, sometimes called D5W, is often used instead if the patient is at risk for having low blood sugar or high sodium.

Some examples of suitable carriers include phosphate-buffered saline or another physiologically acceptable buffer, lactose, dextrose, sucrose, sorbitol, mannitol, starches,

gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. A pharmaceutical composition additionally can include, without limitation, lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. Pharmaceutical compositions can be formulated to provide quick, sustained or delayed release of a mutant reovirus after administration by employing procedures known in the art. In addition to the representative formulations described below, other suitable formulations for use in a pharmaceutical composition can be found in Remington: The Science and Practice of Pharmacy (21th ed.) ed. David B. Troy, Lippincott Williams & Wilkins, 2005. For preparing solid compositions such as tablets, a mutant reovirus can be mixed with a pharmaceutical carrier to form a solid composition. Optionally, tablets or pills can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, a tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

Liquid formulations that include a reovirus and/or other agents for oral administration or for injection generally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. These liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described herein. Such compositions can be administered by the oral or nasal respiratory route for local or systemic effect. Compositions in pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent or intermittent positive pressure breathing machine.

Solution, suspension, or powder compositions may be administered, orally or nasally, from devices which deliver the formulation in an appropriate manner.

Another formulation that is optionally employed in the methods of the present disclosure includes transdermal delivery devices (e.g., patches). Such transdermal patches may be used to provide continuous or discontinuous infusion of a mutant reovirus as described herein. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S. Patent No. 5,023,252. Such patches can be constructed for continuous, pulsatile, or on-demand delivery of mutant reoviruses.

As described above, reoviruses and/or other agents are, if necessary, coated in a liposome or micelle to reduce or prevent an immune response in a mammal that has developed immunity toward a reovirus. Such compositions are referred to as immunoprotected reoviruses and/or agents. See, for example, U.S. Patent Nos. 6,565,831 and 7,014,847. In addition, a mutant reovirus as disclosed herein (e.g., one that lacks or is deficient in sigma3 polypeptide or function) can be proteolytically treated with an enzyme to remove or partially remove any of the other outer capsid proteins present.

In the provided methods, the reovirus is administered in a manner so that it can ultimately contact the target tumor or tumor cells, for example, systemically. The route by which the reovirus is administered, as well as the formulation, carrier or vehicle, depends on the location as well as the type of the target cells. A wide variety of administration routes can be employed. For example, for a solid tumor that is accessible, the reovirus can be administered by injection directly to the tumor. For a hematopoietic tumor, for example, the reovirus can be administered intravenously or intravascularly. For tumors that are not easily accessible within the body, such as metastases, the reovirus is administered in a manner such that it can be transported systemically through the body of the mammal and thereby reach the tumor (e.g., intravenously or intramuscularly). Alternatively, the reovirus can be administered directly to a single solid tumor, where it then is carried systemically through the body to metastases. The reovirus can also be administered subcutaneously, intraperitoneally, intrathecally (e.g., for brain tumor), topically (e.g., for melanoma), orally (e.g., for oral or esophageal cancer), rectally (e.g., for colorectal cancer), vaginally (e.g., for cervical or vaginal cancer), nasally, by inhalation spray or by aerosol formulation (e.g., for lung cancer).

Optionally, the virus is administered continuously to a subject at least once per day or up to throughout the day on consecutive days, for a period of time. Thus, the virus is administered, for example, to subjects by means of intravenous administration in any

pharmacologically acceptable solution, or as an infusion over a period of time. For example, the substance may be administered systemically by injection (e.g., IM or subcutaneously) or taken orally daily at least once per day, or administered by infusion in a manner that results in the daily delivery into the tissue or blood stream of the subject. When the virus is administered by infusion over a period of time, the period of time is, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, or 24 hours, or any time between 1 and 24 hours, inclusive, or more. Optionally, the period of time is 5, 15, 30, 60, 90, 120, 150 or 180 minutes, or any time between 5 and 180 minutes, inclusive, or more. Thus, for example, the virus is administered by infusion for 60 minutes or about 60 minutes. Administrations can be repeated daily for 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 21, 28 days or any number of days between 2 and 28 days, inclusive, or longer.

Therapeutic agents, such as the agents that inhibit production of pro-inflammatory cytokines, of the provided methods are also administered via a wide variety of administration routes. Thus, the agents are administered via any of several routes of administration, including, topically, orally, parenterally, intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, intrahepatically, intracranially, nebulization/inhalation, or by instillation via bronchoscopy. Optionally, the therapeutic agents are administered continuously in the manner set forth in the description above with respect to oncolytic viruses. Thus, for example, the agent is administered, for example, to subjects by means of intravenous administration in any pharmacologically acceptable solution, or as an infusion over a period of time. Optionally, the agents are administered locally at or near the site of the tumor. Alternatively, the agents are administered systemically. The agents that inhibit pro-inflammatory cytokines are administered in an amount sufficient (i.e., an effective amount) to inhibit one or more pro-inflammatory cytokines. By way of example, effective amounts of platinum compounds include from about 5 to 1000 mg/m^2 of tumor volume, or any amount in between 5 and 1000 mg/m^2, inclusive, or more. Thus, for example effective amounts of cisplatin include from about 175-200 mg/m^2 and effective mounts for carboplatin include from about 100-600 mg/m^2. Effective amounts of other agents range from 0.001-10,000 mg/kg body weight or any amount in between 0.001 and 10,000 mg/kg body weight, inclusive. Optionally, effective amounts of platinum compounds include approximately 2 to 7 mg/mL minute (AUC) as calculated by the Calvert formula. Optionally, effective amounts of platinum compounds include approximately 5 or 6 mg/mL minute (AUC) as calculated by the Calvert formula. Optionally, the platinum compounds are administered as an intravenous infusion over a period of 30 minutes.

The reovirus is administered in an amount that is sufficient to treat the proliferative disorder (e.g., an effective amount). A proliferative disorder is treated when administration of a reovirus to proliferating cells affects lysis (e.g., oncolysis) of the affected cells, resulting in a reduction in the number of abnormally proliferating cells, a reduction in the size of a neoplasm, and/or a reduction in or elimination of symptoms (e.g., pain) associated with the proliferating disorder. As used herein, the term oncolysis means at least 10% of the proliferating cells are lysed (e.g., at least about 20%, 30%, 40%, 50%, or 75% of the cells are lysed). The percentage of lysis can be determined, for example, by measuring the reduction in the size of a neoplasm or in the number of proliferating cells in a mammal, or by measuring the amount of lysis of cells *in vitro* (e.g., from a biopsy of the proliferating cells). An effective amount of a virus will be determined on an individual basis and may be based, at least in part, on the particular virus used; the individual's size, age, gender; and the size and other characteristics of the abnormally, proliferating cells. For example, for treatment of a human, approximately 10^3 to 10^{12} plaque forming units (PFU) of a virus are used, depending on the type, size and number of proliferating cells or neoplasms present. The effective amount can be, for example, from about 1.0 PFU/kg body weight to about 10^{15} PFU/kg body weight (e.g., from about 10^2 PFU/kg body weight to about 10^{13} PFU/kg body weight). Optionally, the effective amount is about $1x10^8$ to about $1x10^{12}$ $TCID_{50}$. Optionally, the effective amount is about $1x10^{10}$ $TCID_{50}$.

By way of example, 5-6 mg/ml minute (AUC as calculated by the Calvert formula) of an agent that inhibits pro-inflammatory cytokines, such as carboplatin, is administered to the subject and $1x10^{10}$ $TCID_{50}$ to $3x10^{10}$ $TCID_{50}$ of a reovirus is administered to the subject. Optionally, the agent that inhibits pro-inflammatory cytokines is administered as a thirty minute to one hour intravenous infusion. Optionally, the reovirus is administered as a one hour intravenous infusion.

Optimal dosages of viruses and therapeutic agents and compositions comprising viruses and agents depend on a variety of factors. The exact amount required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disease being treated, the particular virus or vector used and its mode of administration. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the guidance provided herein.

Effective dosages and schedules for administering the compositions may be determined empirically. For example, animal models for a variety of proliferative disorders can be obtained from The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609 USA. Both direct (e.g., histology of tumors) and functional measurements (e.g., survival of a subject or size of a tumor) can be used to monitor response to therapies. These methods involve the sacrifice of representative animals to evaluate the population, increasing the animal numbers necessary for the experiments. Measurement of luciferase activity in the tumor provides an alternative method to evaluate tumor volume without animal sacrifice and allowing longitudinal population-based analysis of therapy.

The dosage ranges for the administration of compositions are those large enough to produce the desired effect in which the symptoms of the disease are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions and anaphylactic reactions. The dosage can be adjusted by the individual physician in the event of any counterindications.

Dosages vary and are administered in one or more dose administrations daily, for one or several days. The provided viruses and therapeutic agents are administered in a single dose or in multiple doses (e.g., two, three, four, six, or more doses). For example, where the administration is by infusion, the infusion can be a single sustained dose or can be delivered by multiple infusions. Treatment may last from several days to several months or until diminution of the disease is achieved.

Combinations of the provided viruses and therapeutic agents are administered either concomitantly (e.g., as an admixture), separately but simultaneously (e.g., via separate intravenous lines into the same subject), or sequentially (e.g., one of the compounds or agents is given first followed by the second). Thus, the term combination is used to refer to concomitant, simultaneous, or sequential administration of two or more agents. By way of example, the agent that inhibits proinflammatory cytokines is administered prior to or at the same time as the oncolytic virus. When one compound is administered prior to another compound, the first compound is administered minutes, hours, days, or weeks prior to administration of the second compound. For example, the first compound can be administered at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24, 36, 48, 60, or 72 hours, or any time between 1 and 72 hours, inclusive, prior to administration of a second compound. Optionally, the first compound is administered more than 72 hours prior to the second compound. By way of another example, the first compound can be administered at 1, 5, 15, 30, 60, 90, or 120 minutes, or any time between 1 and 120

minutes, inclusive, prior to administration of a second compound. Optionally, the first compound is administered at 1, 2, 3, 4, 5, 6, 7, 14, 21, or 28 days, or any amount in between 1 and 28, inclusive, days prior to administration of the second compound. Optionally, the first compound is administered more than 28 days prior to the second compound. For example, the agent that inhibits proinflammatory cytokines is administered from about 1 to 8 hours prior to administration of the oncolytic virus. By way of another example, the agent that inhibits pro-inflammatory cytokines is administered at a time of about one hour prior to administration of the oncolytic virus.

By way of example, one cycle of treatment includes administering the agent that inhibits proinflammatory cytokines and the oncolytic virus includes on day 1. On days 2, 3, 4 and 5, only the oncolytic virus is administered to the subject. Optionally, the subject receives multiple cycles of treatment, for example, two, three, four, five or more cycles of treatment.

Reoviruses or a pharmaceutical composition comprising such reoviruses are optionally packaged into a kit. The kit also includes one or more agents or pharmaceutical compositions comprising such agents that inhibit pro-inflammatory cytokines. It is contemplated that a kit, optionally, also includes one or more chemotherapeutic agents, one or more immunosuppressive agents, and/or one or more anti-antireovirus antibodies. A pharmaceutical composition can be formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of a mutant reovirus calculated to produce the desired therapeutic effect in association with a suitable pharmaceutically acceptable carrier.

It is contemplated that the provided methods may be combined with other tumor therapies such as chemotherapy, radiotherapy, surgery, hormone therapy and/or immunotherapy. Thus, the oncolytic virus may be administered in conjunction with surgery or removal of the neoplasm. Therefore, provided herewith are methods for the treatment of a solid neoplasm comprising surgical removal of the neoplasm and administration of an oncolytic virus at or near to the site of the neoplasm.

It is further contemplated that the compositions in the provided methods are, optionally, administered in conjunction with or in addition to known anticancer compounds or chemotherapeutic agents. Chemotherapeutic agents are compounds which may inhibit the growth of tumors. Such agents, include, but are not limited to. 5-fluorouracil, mitomycin C, methotrexate, hydroxyurea, cyclophosphamide, dacarbazine, mitoxantrone, anthracyclins (Epirubicin and Doxurubicin), antibodies to receptors, such

as herceptin, etopside, pregnasome, hormone therapies such as tamoxifen and anti-estrogens, interferons, aromatase inhibitors, progestational agents and LHRH analogs.

As used herein the terms treatment, treat, treating or ameliorating refers to a method of reducing the effects of a disease or condition or symptom of the disease or condition. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% reduction or amelioration in the severity of an established disease or condition or symptom of the disease or condition. For example, the method for treating cancer is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to control. Thus the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% or any percent reduction in between 10 and 100 as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition or symptoms of the disease or condition.

As used herein, references to decreasing, reducing, or inhibiting include a change of 10, 20, 30, 40, 50 ,60, 70 ,80, 90 percent or greater as compared to a control level. Such terms can include but do not necessarily include complete elimination.

As used herein, the term subject can be a vertebrate, more specifically a mammal (e.g., a human, horse, pig, rabbit, dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent), a fish, a bird or a reptile or an amphibian. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered. As used herein, patient or subject may be used interchangeably and can refer to a subject with a disease or disorder. The term patient or subject includes human and veterinary subjects.

Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if an inhibitor is disclosed and discussed and a number of modifications that can be made to a number of molecules including the inhibitor are discussed, each and every combination and permutation of the inhibitor, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept

applies to all aspects of this disclosure including, but not limited to, steps in methods of using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

A number of aspects have been described. Nevertheless, it will be understood that various modifications may be made. Furthermore, when one characteristic or step is described it can be combined with any other characteristic or step herein even if the combination is not explicitly stated. Accordingly, other aspects are within the scope of the claims.

Examples

Example 1. Anti-tumor Activity of Reovirus and Cisplatin in Mouse Melanoma Model.

Reovirus type 3 Dearing (RV) has demonstrated oncolytic activity in numerous *in vitro* systems, *in vivo* murine models and early clinical trials. To further these studies, the *in vitro* and *in vivo* oncolytic activity of RV in combination with cisplatin (CP), a pseudoalkylating chemotherapeutic which causes DNA cross-linking and is active in a wide range of cancers, was examined. The effect of RV and CP was assessed *in vitro* for synergistic tumor kill and mechanism of tumor death. A synergistic interaction (combination index value (CIV) of less than one) was observed between RV and CP (CIV: ED50, 0.42±0.03; ED75, 0.30±0.02; ED90, 0.24±0.01) on B16.F10 cells. Flow cytometric analysis showed a marked increase in apoptotic cells following combined exposure, compared to single agent exposure.

For *in vivo* evaluation, subcutaneous B16.F10 tumors in C57Bl/6 mice or K1735 tumors in C3H mice were treated with intratumoral (i.t.) RV and intraperitoneal (i.p.) CP either alone or in combination. Tumor volume was estimated thrice weekly. Tumors and organs were harvested post-treatment for viral retrieval and histology; serum samples were tested for cytokine production and induction of neutralising anti-reovirus antibody (NARA). Figures 1A, 1B, 1C and 1D show reduced tumor growth and increased survival following reovirus/cisplatin combination therapy. C57Bl/6 (Figures 1A and 1C) and C3H (Figures 1B and 1D) mice bearing subcutaneous B16.F10 and K1735 tumors respectively

were treated on days 1 and 4 with either reovirus alone i.t. (squares), cisplatin alone i.p. (triangles), or reovirus and cisplatin in combination (circles). Control treated mice (diamonds) received PBS. Tumors were measured on the days indicated and tumor volume expressed as tumor volume relative to volume at commencement of treatment (Figures 1A and 1B). Mice were euthanized when tumors exceeded 15mm in any one dimension. Survival is expressed as Kaplan-Myer plots (Figures 1C and 1D). These data show reduced tumor growth and extended median survival time was observed in mice treated with RV/CP combination therapy compare to single agent treatments (Figures 1A, 1B, 1C and 1D). Mean relative tumor volumes ±SD at day 12 were, control: all reached endpoint, RV alone: 8.92±6.94, CP alone: 9.87±2.80, RV plus CP: 3.86±2.24. Median survival (days) were, control: 6, RV: 12, CP: 8, combination of RV and CP: 17. Live virus was recovered from the tumors of all RV only treated animals and from the liver and heart of 1/6 mice. In contrast live virus was detected in only 50% of tumors from combination treated mice but in the liver of 4/6 mice. CP did not affect the neutralizing anti-reovirus antibody (NARA) response to RV (Figure 2), but caused a marked attenuation of production of proinflammatory cytokines to RV when used in combination (Figures 3A, 3B, 3C, 3D, 3E, 3F, and 3G).

Taken together, these results show that the addition of chemotherapeutic agents can significantly enhance the anti-tumor efficacy of RV therapy. Furthermore, a reduction in viral inflammatory responses in vital organs by concomitant chemotherapy may allow more intensive dosing schedules to increase overall efficacy of the reovirus.

Example 2. Reovirus and Carboplatin Protocols for Humans.

This is a study design of reovirus given intravenously with carboplatin every 3 weeks.

Carboplatin is administered as a 30 minute intravenous infusion at a dose calculated by the Calvert formula (AUC 5 mg/mL minute or 6 mg/mL minute with GFR measured by 51Cr EDTA). Reovirus is then administered as a 1 hour intravenous infusion at a dose of $1x10^{10}$ or $3x10^{10}$ $TCID_{50}$.

On days 2 through 5, only reovirus will be administered, using the same dose and method as used on Day 1.

Table 2 - Dosing Methods

	Carboplatin Dose AUC mg/mL min Day 1 only	Reovirus dose ($TCID_{50}$) Days 1 - 5
Method 1	5	$1x10^{10}$
Method 2	5	$3x10^{10}$
Method 3	6	$1x10^{10}$
Method 4	6	$3x10^{10}$

WHAT IS CLAIMED IS:

1. Use of one or more reoviruses and

one or more platinum compounds that inhibit expression or activity of a pro-inflammatory cytokine but do not inhibit production of neutralizing anti-reovirus antibodies (NARA) in the manufacture of a medicament for treating a proliferative disorder in a subject.

2. The use of claim 1, wherein approximately 10^3 to 10^{12} plaque forming units (PFU) of the oncolytic virus is to be administered to the subject.

3. The use of claim 2, wherein approximately 10^8 to 10^{12} plaque forming units (PFU) of the oncolytic virus is to be administered to the subject.

4. The use of claim 1, wherein approximately 10^8 to 10^{12} $TCID_{50}$ of the oncolytic virus is to be administered to the subject.

5. The use of claim 1, wherein approximately 5 to 1000 mg/m^2 of the agent that inhibits proinflammatory cytokines is to be administered to the subject.

6. The use of claim 1, wherein approximately 0.001-10,000 mg/kg body weight of the agent that inhibits proinflammatory cytokines is to be administered to the subject.

7. The use of claim 1, wherein 2 to 7 mg/mL minute (AUC) of the agent that inhibits proinflammatory cytokines is to be administered to the subject.

8. The use of claim 1, wherein the platinum compound is selected from the group consisting of cisplatin, carboplatin and oxaliplatin.

9. The use of claim 8, wherein approximately 175-200 mg/m^2 of the cisplatin is to be administered to the subject.

10. The use of claim 8, wherein approximately 200-600 mg/m^2 of the carboplatin is to be administered to the subject.

11. The use of claim 8, wherein 5 or 6 mg/mL minute (AUC) of the carboplatin is to be administered to the subject.

12. The use of any one of claims 1-11, wherein the reovirus is a mammalian reovirus or a human reovirus.

13. The use of claim 12, wherein the human reovirus is selected from the group consisting of serotype 1 reovirus, serotype 2 reovirus and serotype 3 reovirus.

14. The use of claim 12, wherein the human reovirus is serotype 3 reovirus.

15. The use of claim 12, wherein the human reovirus has IDAC Accession No. 190907-01.

16. The use of any one of claims 1-11, wherein the agent that inhibits pro-inflammatory cytokines is to be administered at the same time, before or after the reovirus.

17. The use of claim 16, wherein the agent that inhibits pro-inflammatory cytokines is to be administered at the same time as the reovirus.

18. The use of claim 16, wherein the agent that inhibits pro-inflammatory cytokines is to be administered before the reovirus.

19. The use of claim 18, wherein the agent is to be administered from 1 to 12 hours before the oncolytic virus.

20. The use of claim 18, wherein the agent is to be administered from 1 to 60 minutes before the oncolytic virus.

21. The use of claim 1, wherein the reovirus is to be administered in multiple doses.

22. The use of claim 21, wherein the agent that inhibits pro-inflammatory cytokines is to be administered once.

23. The use of claim 21, wherein the agent that inhibits pro-inflammatory cytokines is to be administered in multiple doses.