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(54) **Title:** METHODS FOR TREATING OR PREVENTING EBOLAVIRUS OR MARBURGVIRUS INFECTIONS

(57) **Abstract:** Described herein are methods of treating and preventing infections with a filovirus, such as Ebola virus or Marburg virus, comprising administering an effective amount of apilimod or a salt thereof.



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**METHODS FOR TREATING OR PREVENTING *EBOLAVIRUS* OR
MARBURGVIRUS INFECTIONS**

RELATED APPLICATION

This application claims the benefit of priority to U.S. Provisional Patent Application
5 serial number 62/100,678, filed January 7, 2015. This application is hereby incorporated
herein by reference in its entirety.

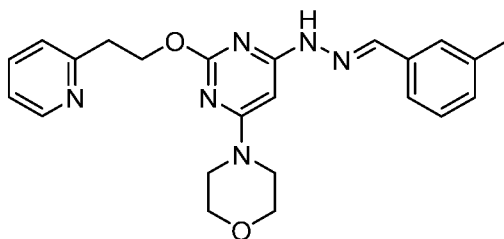
STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under grant numbers U54
AI057159 and U19 AI109740 awarded by the National Institutes of Health. The
10 government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Viruses classified in the genera *Ebolavirus* or *Marburgvirus*, such as *Zaire
ebolavirus* (EboV), are highly pathogenic enveloped viruses that can cause outbreaks of
zoonotic infection in humans. EboV is transmitted by close contact and virus levels increase
15 by 75-fold/day for several days after initial infection. The clinical symptoms are
manifestations of the massive production of pro-inflammatory cytokines in response to
infection and in many outbreaks, mortality exceeds 75%. The endothelial cell dysfunction
associated with “cytokine storm” results in capillary leak, hypovolemic shock, disseminated
intravascular coagulation, and inadequate perfusion of major organs. The unpredictable
20 onset, ease of transmission, rapid progression of disease, high mortality, and lack of
effective vaccine or therapy have created a high level of public concern about EboV.
Current therapy is supportive; there is no effective anti-EboV vaccine or therapy. Therefore,
development of anti-EboV drugs is a high priority.

Apilimod ((*E*)-4-(6-(2-(3-methylbenzylidene)hydrazinyl)-2-(2-(pyridin-2-yl)ethoxy)
25 pyrimidin-4-yl)morpholine)) (below) is a small molecule compound developed to
specifically block toll-like receptor-mediated (TLR-mediated) IL-12/IL-23 production. It
has been tested in patients with Crohn’s disease (CD), rheumatoid arthritis (RA), and
psoriasis. Although apilimod showed clinical improvement in patients with active CD in a
phase I/IIA trial, no significant improvement over placebo was seen in a phase II trial,
30 though it was generally well tolerated.



apilimod

Apilimod specifically binds to and inhibits the activity of phosphatidylinositol-3-phosphate 5-kinase (PIKfyve) (Cai, X., et al. Chem. Biol. 2013, 20, 912-921). PIKfyve is a 240 kDa lipid kinase that phosphorylates the D-5 position in endosomal phosphatidylinositol-3-phosphate (PI3P) to yield the 3,5-bisphosphate (PI(3,5)P₂). This kinase binds to PI(3)P via its FYVE domain. PIKfyve is critical for maintaining the proper morphology of the endosome/lysosome. An enlarged endosome/lysosome structure has been observed in cells expressing PIKfyve dominant negative or siRNA. Vac14 and Sac3 form a regulatory complex with PIKfyve to control endosomal phosphoinositide metabolism. The vacuolization and low PI(3,5)P₂ levels in fibroblasts isolated from Vac14 and Sac3 null mice suggest that both are required for maximal PIKfyve activity. PIKfyve-mediated PI(3,5)P₂ signaling regulates endosomal trafficking and plays a key role in multiple biological processes, such as GLUT4 translocation, retroviral budding, and TLR-mediated cytokine expression.

The present invention addresses the need for methods for treating subjects infected with viruses of the genera *Ebolavirus* or *Marburgvirus*, or who are at risk for infection with such viruses.

SUMMARY OF THE INVENTION

In certain embodiments, the invention relates to a method of treating or preventing a viral infection in a subject in need thereof comprising administering to the subject an effective amount of apilimod, or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer, or stereoisomer thereof, wherein the viral infection is caused by a virus classified in of the genera *Ebolavirus* or *Marburgvirus*.

In certain embodiments, the invention relates to any of the methods described herein, wherein the subject is a subject having a viral infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to treat the viral infection.

In certain embodiments, the invention relates to any of the methods described herein, wherein the subject is a subject at risk of having a viral infection caused by a virus

classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to prevent viral infection of the subject.

In certain embodiments, the invention relates to any of the methods described herein, wherein the virus is *Zaire ebolavirus*, *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*.

In certain embodiments, the invention relates to any of the methods described herein, wherein the virus is *Zaire ebolavirus*.

In certain embodiments, the invention relates to any of the methods described herein, wherein the virus is *Marburg marburgvirus*.

10 In certain embodiments, the invention relates to any of the methods described herein, wherein the subject is human.

In certain embodiments, the invention relates to any of the methods described herein, wherein apilimod is administered intravenously, intraperitoneally, or subcutaneously.

15 In certain embodiments, the invention relates to any of the methods described herein, wherein apilimod is administered continuously.

In certain embodiments, the invention relates to any of the methods described herein, wherein apilimod is administered continuously using a pump device.

20 In certain embodiments, the invention relates to any of the methods described herein, wherein apilimod is administered as a salt. In certain embodiments, apilimod mesylate is administered.

In certain embodiments, the invention relates to a method of inhibiting PIKfyve in a cell that has been infected with or is at risk of being infected with a virus classified in the genera *Ebolavirus* or *Marburgvirus* comprising contacting the cell with an effective amount
25 of apilimod, or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer, or stereoisomer thereof.

In certain embodiments, the invention relates to any of the methods described herein, wherein the cell has been infected with a virus classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to treat the
30 infection of the cell.

In certain embodiments, the invention relates to any of the methods described herein, wherein the cell is at risk of being infected with a virus classified in the genera

Ebolavirus or *Marburgvirus*; and the effective amount of apilimod is an amount effective to prevent infection of the cell.

In certain embodiments, the invention relates to any of the methods described herein, wherein the virus is *Zaire ebolavirus*, *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*.

In certain embodiments, the invention relates to any of the methods described herein, wherein the virus is *Zaire ebolavirus*.

In certain embodiments, the invention relates to any of the methods described herein, wherein the virus is *Marburg marburgvirus*.

10 In certain embodiments, the invention relates to any of the methods described herein, wherein the cell is a human cell.

In certain embodiments, the invention relates to any of the methods described herein, wherein the cell is in vitro.

15 In certain embodiments, the invention relates to any of the methods described herein, wherein the IC₅₀ of apilimod is less than about 50 nM, less than about 40 nM, less than about 30 nM, or less than about 20 nM.

In certain embodiments, the invention relates to any of the methods described herein, wherein the cell is in vivo.

20 In certain embodiments, the invention relates to any of the methods described herein, wherein the cell is a liver cell.

In certain embodiments, the invention relates to any of the methods described herein, wherein the cell is a hepatocyte.

BRIEF DESCRIPTION OF THE DRAWINGS

25 **Figure 1** depicts the inhibition by apilimod at various concentrations of single cycle entry of MLV particles pseudotyped with EboV, MARV, SARS, and MERS glycoproteins.

Figure 2 depicts the inhibition by apilimod at various concentrations (left bar = DMSO control; second bar from left = 25 nM apilimod; third bar = 0.1 μM apilimod; fourth bar = 0.25 μM apilimod; fifth bar = 0.50 μM apilimod; right bar = 1.0 μM apilimod) of single cycle entry of MLV particles pseudotyped with ZEboV, SEboV, CIEboV, REboV, 30 BEboV, MarV, or LFV glycoproteins.

Figure 3 depicts the inhibition by CQ at 10 μM (left panel), Compound 10 at 1 μM (center panel), and apilimod at 1 μM (right panel) of single cycle entry of MLV particles

pseudotyped with ZEboV, MarV, LFV, VSV, NiV, LCMV, MLV, Flu, or SARS glycoproteins.

Figure 4A depicts the inhibition by apilimod at various concentrations of single cycle entry into Vero cells of MLV particles pseudotyped with GP from a mouse-adapted strain of EboV (squares) and of MLV particles pseudotyped with GP from ZEboV (diamonds).

Figure 4B depicts the inhibition by apilimod at various concentrations of single cycle entry into primary mouse embryo fibroblast cells of MLV particles pseudotyped with GP from a mouse-adapted strain of EboV (squares) and of MLV particles pseudotyped with GP from ZEboV (diamonds).

Figure 5 depicts the inhibition by apilimod at various concentrations of cells infected with Ebolavirus (diamonds) or Marburg virus (squares). The CC_{50} is also depicted (triangles).

Figure 6A depicts a schematic representation of an assay to localize the action of various EboV inhibitors.

Figure 6B depicts images of cells incubated with red-labeled dextran, an inhibitor of EboV or DMSO control, and green-labeled dextran. The left image depicts the control cells; the middle image depicts cells exposed to a NPC1 inhibitor, and the right image depicts cells exposed to a second EboV inhibitor, Compound 10.

Figure 7 depicts the correlation between the anti-EboV activity of EboV inhibitors and their activity in blocking lysosomal degradation marker protein LC3-II.

Figure 8 depicts the steady state level of lysosome trafficking marker LC3-II after exposure to apilimod.

Figure 9A depicts a schematic representation of an experiment designed to identify the protein target of EboV inhibitors.

Figure 9B depicts the results of the experiment depicted in Figure 9A.

Figure 10 depicts the results of experiments designed to elucidate the target of certain EboV inhibitors.

Figure 11 depicts the inhibition by Compound 10 of single cycle entry of MLV particles pseudotyped with ZEboV, SARS, or LFV glycoproteins into wild type HT1080 cells (left panel) or cells in which the Fig4 subunit of the PIP complex had been deleted (right panel).

DETAILED DESCRIPTION OF THE INVENTION

Overview

In certain embodiments, the invention relates to the use of apilimod as a specific and potent inhibitor of viral infection in a subject in need thereof, wherein the viral infection is caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*. In certain embodiments, the viral infection is selected from the group consisting of Ebola virus (EBOV or ZEBOV), Marburg virus (MARV), Ravn virus (RAVV), Bundibugyo virus (BDBV or BEBOV), Reston virus (RESTV or REBOV), Sudan virus (SUDV or SEBOV), and Tai Forest virus (TAFV or CIEBOV). In certain embodiments, the virus is *Zaire ebolavirus*, *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*. Viral infections of this type are characterized by hemorrhagic fever, including abnormalities in blood coagulation.

To treat a subject with a viral infection means to reduce or stop the spread of virus in the subject, or to eliminate the virus from the subject, or to reduce or eliminate a sign or symptom of viral infection in the subject.

Subjects who are at risk for infection with these viruses, or subjects in need, include subjects who have been exposed to a virus classified in the genera *Ebolavirus* or *Marburgvirus* or are at risk of exposure to one of these viruses. In addition to the natural occurrence of viruses classified in the genera *Ebolavirus* or *Marburgvirus*, there is the potential for exposure to these pathogens if they are used as agents of bioterrorism or biological warfare.

In certain embodiments, administration of apilimod to the subject in need thereof would normally be limited to periods when the subject either has a viral infection or when the subject has been exposed to a virus classified in the genera *Ebolavirus* or *Marburgvirus* or is at risk of exposure to one of these viruses, in order to minimize any deleterious effect of administration of the agent. Ebola/marburgvirus infections are typically acute in nature, so drug treatment of infection for only a short period of time may be appropriate.

In certain embodiments, apilimod can be administered to the subject in a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

Definitions

The articles “a” and “an” are used herein 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.

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more”
5 of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another
10 embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least
15 one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e., “one or the other
20 but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one
25 element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers,
30 whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including

elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

5 It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

10 In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

15 The term "viral infection" as used herein refers to infection by a viral pathogen wherein there is clinical evidence of the infection based on symptoms or based on the demonstration of the presence of the viral pathogen in a biological sample from the individual. As used herein an "individual" refers to an animal, preferably a mammal, including both non-human mammals and humans, and more preferably, refers to a human.

20 The expression "effective amount" when used to describe therapy to an individual suffering from a viral infection refers to the amount of a compound that results in a therapeutically useful effect on the symptoms of the viral infection and/or a reduction in viral load.

"Treatment of a viral infection" as used herein encompasses alleviating, reducing
25 the frequency of, or eliminating one or more symptoms of the infection and/or reducing the viral load.

Apilimod

In certain embodiments, the invention relates to the use of apilimod as a specific, selective, and potent inhibitor of a viral infection, wherein the viral infection is caused by a
30 virus classified in the genera *Ebolavirus* or *Marburgvirus*. In certain embodiments, the viral infection is selected from the group consisting of Ebola virus (EBOV or ZEBOV), Marburg virus (MARV), Ravn virus (RAVV), Bundibugyo virus (BDBV or BEBOV), Reston virus (RESTV or REBOV), Sudan virus (SUDV or SEBOV), and Tai Forest virus (TAFV or

CIEBOV). In certain embodiments, the viral infection is caused by *Zaire ebolavirus*, *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*.

In certain embodiments, apilimod may be provided as a salt with pharmaceutically compatible counterions (*i.e.*, pharmaceutically acceptable salts). A “pharmaceutically acceptable salt” means any non-toxic salt that, upon administration to a recipient, is capable of providing, either directly or indirectly, apilimod or a prodrug of apilimod. A “pharmaceutically acceptable counterion” is an ionic portion of a salt that is not toxic when released from the salt upon administration to a recipient. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

Acids commonly employed to form pharmaceutically acceptable salts include inorganic acids such as hydrogen bisulfide, hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, salicylic, tartaric, bitartaric, ascorbic, maleic, besylic, fumaric, gluconic, glucuronic, formic, glutamic, methanesulfonic, ethanesulfonic, benzenesulfonic, lactic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic and acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caprate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, terephthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, β -hydroxybutyrate, glycolate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like salts. Preferred pharmaceutically acceptable acid addition salts include those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and especially those formed with organic acids such as maleic acid.

In certain embodiments, apilimod may be provided as its methanesulfonate salt (“apilimod mesylate”).

Suitable bases for forming pharmaceutically acceptable salts with acidic functional groups include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or trialkylamines; dicyclohexylamine; tributyl amine; pyridine; N-methyl-N-ethylamine; diethylamine; triethylamine; mono-, bis-, or tris-(2-hydroxy-lower alkyl amines), such as mono-, bis-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxy lower alkyl)-amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine, or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like.

Apilimod and its salts may exist in more than one crystal form and the present invention includes methods of using each crystal form and mixtures thereof.

Apilimod and its salts may also exist in the form of solvates, for example hydrates, and the present invention includes methods of using each solvate and mixtures thereof.

Apilimod may exist in different tautomeric forms or as different geometric isomers, and the present invention includes methods of using each tautomer and/or geometric isomer of apilimod and mixtures thereof.

Apilimod may exist in different stable conformational forms which may be separable. Torsional asymmetry due to restricted rotation about an asymmetric single bond, for example because of steric hindrance or ring strain, may permit separation of different conformers. The present invention includes methods of using each conformational isomer of apilimod and mixtures thereof.

The present invention also includes methods of using pro-drugs of apilimod. As used herein the term "pro-drug" refers to an agent that is converted into the parent drug *in vivo* by some physiological chemical process (e.g., a prodrug on being brought to the physiological pH is converted to the desired drug form). Pro-drugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmacological compositions over the parent drug. Pro-drugs have many useful properties. For example, a pro-drug may be more water soluble than the ultimate drug, thereby facilitating intravenous administration of the drug. A pro-drug may also have a higher level of oral bioavailability than the ultimate drug. After

administration, the prodrug is enzymatically or chemically cleaved to deliver the ultimate drug in the blood or tissue.

Exemplary pro-drugs release an amine of apilimod wherein the free hydrogen of an amine substituent is replaced by (C₁-C₆)alkanoyloxymethyl, 1-((C₁-C₆)alkanoyloxy)ethyl, 1-methyl-1-((C₁-C₆)alkanoyloxy)ethyl, (C₁-C₆)alkoxycarbonyl-oxymethyl, N-(C₁-C₆)alkoxycarbonylamino-methyl, succinoyl, (C₁-C₆)alkanoyl, α -amino(C₁-C₄)alkanoyl, α -aminoacyl, or α -aminoacyl- α -aminoacyl wherein said α -aminoacyl moieties are independently any of the naturally occurring L-amino acids found in proteins, -P(O)(OH)₂, -P(O)(O(C₁-C₆)alkyl)₂ or glycosyl (the radical resulting from detachment of the hydroxyl of the hemiacetal of a carbohydrate).

The term "chemically protected form," as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group). It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form.

By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts, Wiley, 1991), and Protective Groups in Organic Synthesis (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).

For example, an amine group may be protected, for example, as an amide (-NRC(=O)R) or a urethane (-NRC(=O)OR), for example, as: a methyl amide (-NHC(=O)CH₃); a benzyloxy amide (-NHC(=O)OCH₂C₆H₅NHCbz); as a t-butoxy amide (-NHC(=O)OC(CH₃)₃, -NHBoc); a 2-biphenyl-2-propoxy amide (-NHC(=O)OC(CH₃)₂C₆H₄C₆H₅NHBoc), as a 9-fluorenylmethoxy amide (-NHFmoc), as a 6-nitroveratryloxy amide (-NHNVoc), as a 2-trimethylsilylethyloxy amide (-NHTEoc), as a 2,2,2-trichloroethyloxy amide (-NHTroc), as an allyloxy amide (-NHAlloc), as a 2-(phenylsulfonyl)ethyloxy amide (-NHPsec); or, in suitable cases (e.g., cyclic amines), as a nitroxide radical.

Methods

In certain embodiments, the invention relates to a method of treating or preventing a viral infection in a subject in need thereof comprising administering to the subject an

effective amount of apilimod, or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof, wherein the viral infection is caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*. In certain embodiments, the viral infection is selected from the group consisting of Ebola virus (EBOV or ZEBOV), Marburg virus (MARV), Ravn virus (RAVV), Bundibugyo virus (BDBV or BEBOV), Reston virus (RESTV or REBOV), Sudan virus (SUDV or SEBOV), and Tai Forest virus (TAFV or CIEBOV). In certain embodiments, the viral infection is caused by *Zaire ebolavirus*, *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*. In certain embodiments, the viral infection is caused by *Zaire ebolavirus* or *Marburg marburgvirus*.

The methods of the invention are useful for treating a subject in need thereof. A subject in need thereof is a subject having or at risk of having an infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*. In its broadest sense, the terms "treatment" or "to treat" refer to both therapeutic and prophylactic treatments. If the subject in need of treatment is experiencing a condition (i.e., has or is having a particular condition), then "treating the condition" refers to ameliorating, reducing or eliminating one or more symptoms arising from the condition. If the subject in need of treatment is one who is at risk of having a condition, then treating the subject refers to reducing the risk of the subject having the condition or, in other words, decreasing the likelihood that the subject will develop an infectious disease, as well as to a treatment after the subject has been infected in order to fight the infectious disease, e.g., reduce or eliminate it altogether or prevent it from becoming worse.

Thus the invention encompasses the use of apilimod alone or in combination with other therapies (e.g., therapeutics), for the treatment of a subject having or at risk of having an infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*. A "subject having an infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*" is a subject that has had contact with a virus classified in the genera *Ebolavirus* or *Marburgvirus*, wherein the virus has invaded the body of the subject. The word "invade" as used herein refers to contact by the virus with an external surface of the subject, e.g., skin or mucosal membranes and/or refers to the penetration of the external surface of the subject by the virus. A "subject at risk of having an infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*" is one that has been exposed to or may become exposed to a virus classified in the genera *Ebolavirus* or *Marburgvirus*, or a

subject in a geographical area in which an infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus* has been reported. Further risks include close contact with a human or non-human primate or their tissues infected with the virus. Such persons include laboratory or quarantine facility workers who handle non-human primates that have
5 been associated with the disease. In addition, hospital staff and family members who care for patients with the disease are at risk if they do not use proper barrier nursing techniques.

As used herein, a subject includes humans and non-human animals such as non-human primates, dogs, cats, sheep, goats, cows, pigs, horses and rodents.

In certain embodiments, the viral infection is caused by *Zaire ebolavirus*. Infection
10 by Ebola virus leads to Ebola Hemorrhagic Fever (EHF), the clinical manifestations of which are severe. The incubation period varies between four and sixteen days. The initial symptoms are generally a severe frontal and temporal headache, generalized aches and pains, malaise, and by the second day the victim will often have a fever. Later symptoms include watery diarrhea, abdominal pain, nausea, vomiting, a dry sore throat, and anorexia.
15 By day seven of the symptoms, the patient will often have a maculopapular (small slightly raised spots) rash. At the same time the person may develop thrombocytopenia and hemorrhagic manifestations, particularly in the gastrointestinal tract, and the lungs, but it can occur from any orifice, mucous membrane or skin site. Ebola causes lesions in almost every organ, although the liver and spleen are the most noticeably affected. Both are
20 darkened and enlarged with signs of necrosis. The cause of death (>75% in most outbreaks) is normally shock, associated with fluid and blood loss into the tissues. The hemorrhagic and connective tissue complications of the disease are not well understood, but may be related to onset of disseminated intravascular coagulation.

In certain embodiments, the viral infection is caused by *Marburg marburgvirus*. As
25 used herein, the term "Marburg virus" refers to the filovirus that causes Marburg hemorrhagic fever. Marburg hemorrhagic fever is a rare, severe type of hemorrhagic fever which affects both humans and non-human primates. The case-fatality rate for Marburg hemorrhagic fever is estimated to be about 70%. After an incubation period of 5-10 days, the onset of the disease is sudden and is marked by fever, chills, headache, and myalgia.
30 Around the fifth day after the onset of symptoms, a maculopapular rash, most prominent on the trunk (chest, back, stomach), may occur. Nausea, vomiting, chest pain, a sore throat, abdominal pain, and diarrhea then may appear. Symptoms become increasingly severe and

may include jaundice, inflammation of the pancreas, severe weight loss, delirium, shock, liver failure, massive hemorrhaging, and multi-organ dysfunction.

Apilimod and pharmaceutical compositions comprising apilimod are delivered in effective amounts. The term "effective amount" refers to the amount necessary or sufficient to realize a desired biologic effect. Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial toxicity and yet is effective to treat the particular subject. In addition, based on testing, toxicity of apilimod is expected to be low. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular inhibitor being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of apilimod and/or other therapeutic agent without necessitating undue experimentation. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to some medical judgment. Multiple doses per day may be contemplated to achieve appropriate systemic levels of compounds. Appropriate systemic levels can be determined by, for example, measurement of the patient's peak or sustained plasma level of the drug. "Dose" and "dosage" are used interchangeably herein.

The therapeutically effective amount can be initially determined from preliminary in vitro studies and/or animal models. A therapeutically effective dose can also be determined from human data for inhibitors that have been tested in humans and for compounds that are known to exhibit similar pharmacological activities, such as other related active agents. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods well-known in the art, is well within the capabilities of the ordinarily skilled artisan.

In certain embodiments, an effective amount of apilimod to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer apilimod until a dosage is

reached that achieves the desired effect. In certain embodiments, depending on the type and severity of the infection, about 10 ng/kg to 2000 mg/kg of apilimod is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. For repeated administrations over several days
5 or longer, the treatment is sustained until a desired suppression of disease symptoms occurs or reduction of viral load is achieved. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. In certain embodiments, a typical dosage for systemic treatment might range from about 10 ng/kg to up to 2000 mg/kg or more, depending on the factors mentioned above.

10 Administering inhibitors of certain enzymes for the treatment or prevention of a filovirus is discussed in U.S. Patent Application Publication No. US 2014-0018338, which is hereby incorporated by reference in its entirety.

In certain embodiments, the invention relates to a method of inhibiting PIKfyve in a cell that has been infected with or is at risk of being infected with a virus classified in the
15 genera *Ebolavirus* or *Marburgvirus* comprising contacting the cell with an effective amount of apilimod, or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer, or stereoisomer thereof. In certain embodiments, these methods are particularly useful because it targets cellular proteins, such as PIKfyve, rather than viral proteins; treating or preventing viral infections in this manner decreases the likelihood that
20 the viruses will develop drug resistance.

Combination Therapy

In certain embodiments, apilimod can be combined with other therapeutic agents. Apilimod and other therapeutic agent may be administered simultaneously or sequentially. When the other therapeutic agents are administered simultaneously they can be
25 administered in the same or separate formulations, but are administered at the same time. The other therapeutic agents are administered sequentially with one another and with apilimod when the administration of the other therapeutic agents and apilimod is temporally separated. The separation in time between the administration of these compounds may be a matter of minutes or it may be longer. Other therapeutic agents include but are not limited
30 to anti-viral vaccines and anti-viral agents. In some instances apilimod is administered with multiple therapeutic agents, i.e., 2, 3, 4 or even more different anti-viral agents.

An anti-viral vaccine is a formulation composed of one or more viral antigens and one or more adjuvants. The viral antigens include proteins or fragments thereof as well as whole killed virus. Adjuvants are well known to those of skill in the art.

Antiviral agents are compounds that prevent infection of cells by viruses or
5 replication of the virus within the cell. There are many fewer antiviral drugs than antibacterial drugs because viruses are more dependent on host cell factors than bacteria. There are several stages within the process of viral infection which can be blocked or inhibited by antiviral agents. These stages include, attachment of the virus to the host cell (immunoglobulin or binding peptides), membrane penetration inhibitors, e.g. T-20,
10 uncoating of the virus (e.g. amantadine), synthesis or translation of viral mRNA (e.g. interferon), replication of viral RNA or DNA (e.g. nucleotide analogues), maturation of new virus proteins (e.g. protease inhibitors), and budding and release of the virus.

Nucleotide analogues are synthetic compounds that are similar to nucleotides, but which have an incomplete or abnormal deoxyribose or ribose group. Once the nucleotide
15 analogues are in the cell, they are phosphorylated, producing the triphosphate form, which competes with normal nucleotides for incorporation into the viral DNA or RNA. Once the triphosphate form of the nucleotide analogue is incorporated into the growing nucleic acid chain, it causes irreversible association with the viral polymerase and thus chain termination. Nucleotide analogues include, but are not limited to, acyclovir (used for the
20 treatment of herpes simplex virus and varicella-zoster virus), gancyclovir (useful for the treatment of cytomegalovirus), idoxuridine, ribavirin (useful for the treatment of respiratory syncytial virus), dideoxyinosine, dideoxycytidine, zidovudine (azidothymidine), imiquimod, and resiquimod.

The interferons are cytokines that are secreted by virus-infected cells as well as
25 immune cells. The interferons function by binding to specific receptors on cells adjacent to the infected cells, causing a change in the cell that protects it from infection by the virus. α - and β -interferon also induce the expression of Class I and Class II MHC molecules on the surface of infected cells, resulting in increased antigen presentation for host immune cell recognition. α - and β -interferons are available as recombinant forms and have been used for
30 the treatment of chronic hepatitis B and C infection. At the dosages that are effective for anti-viral monotherapy, interferons have severe side effects such as fever, malaise and weight loss.

Anti-viral agents that may be useful in combination with apilimod include but are not limited to immunoglobulins, amantadine, interferons, nucleotide analogues, and other protease inhibitors (other than the papain-like cysteine protease inhibitors--although combinations of papain-like cysteine protease inhibitors are also useful). Specific examples of anti-viral agents include but are not limited to Acemannan; Acyclovir; Acyclovir Sodium; Adefovir; Alovudine; Alvircept Sudotox; Amantadine Hydrochloride; Aranotin; Arildone; Ateviridine Mesylate; Avridine; Cidofovir; Cipamfylline; Cytarabine Hydrochloride; Delavirdine Mesylate; Desciclovir; Didanosine; Disoxaril; Edoxudine; Enviroxime; Famciclovir; Famotone Hydrochloride; Fiacitabine; Fialuridine; Fosarilate; Foscarnet Sodium; Fosfonet Sodium; Ganciclovir; Ganciclovir Sodium; Idoxuridine; Kethoxal; Lamivudine; Lobucavir; Memotone Hydrochloride; Methisazone; Nevirapine; Penciclovir; Pirodavis; Ribavirin; Rimantadine Hydrochloride; Saquinavir Mesylate; Somantadine Hydrochloride; Sorivudine; Statolon; Stavudine; Tilorone Hydrochloride; Trifluridine; Valacyclovir Hydrochloride; Vidarabine; Vidarabine Phosphate; Vidarabine Sodium Phosphate; Viroxime; Zalcitabine; Zidovudine; and Zinviroxime.

Immunoglobulin therapy is used for the prevention of viral infection. Immunoglobulin therapy for viral infections is different than for bacterial infections, because rather than being antigen-specific, the immunoglobulin therapy functions by binding to extracellular virions and preventing them from attaching to and entering cells that are susceptible to the viral infection. The therapy is useful for the prevention of viral infection for the period of time that the antibodies are present in the host. In general there are two types of immunoglobulin therapies, normal immunoglobulin therapy and hyper-immunoglobulin therapy. Normal immune globulin therapy utilizes an antibody product, which is prepared from the serum of normal blood donors and pooled. This pooled product contains low titers of antibody to a wide range of human viruses, such as hepatitis A, parvovirus, enterovirus (especially in neonates). Hyper-immune globulin therapy utilizes antibodies which are prepared from the serum of individuals who have high titers of an antibody to a particular virus. Those antibodies are then used against a specific virus. Another type of immunoglobulin therapy is active immunization. This involves the administration of antibodies or antibody fragments to viral surface proteins.

Pharmaceutical Compositions

The pharmaceutical compositions or formulations of the invention are administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, 5 adjuvants, and optionally other therapeutic ingredients.

For use in therapy, an effective amount of apilimod can be administered to a subject by any mode that delivers the inhibitor to the desired surface. Administering the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. Preferred routes of administration include but are not limited 10 to oral, intrathecal, intra-arterial, direct bronchial application, parenteral (e.g. intravenous), intramuscular, intranasal, sublingual, intratracheal, inhalation, ocular, vaginal, and rectal, e.g., using a suppository.

In certain embodiments, lyophilized formulations and liquid formulations of apilimod suitable for injection are particularly efficacious. Suitable dosage forms for use in 15 embodiments of the invention encompass physiologically/pharmaceutically acceptable carriers that are inherently non-toxic and non-therapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes 20 such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, P6N (Neumedicines, Pasadena, Calif.) and PEG. Carriers for topical or gel-based forms include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene- 25 polyoxypropylene-block polymers, PEG, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations.

Administration can be performed by use of an indwelling catheter and a continuous 30 administration means such as a pump, or it can be administered by implantation, e.g., implantation of a sustained-release vehicle. More specifically, apilimod can be injected through chronically implanted cannulas or chronically infused with the help of osmotic minipumps. Subcutaneous pumps are available that deliver active agents through a small

tubing. Highly sophisticated pumps can be refilled through the skin and their delivery rate can be set without surgical intervention. Suitable administration protocols and delivery systems involving a subcutaneous pump device or continuous infusion through a totally implanted drug delivery system are known to those skilled in the art.

5 Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), poly(vinylalcohol), polylactides, copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, 10 degradable lactic acid-glycolic acid copolymers (such as injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

15 Pharmaceutical formulations are prepared for storage by mixing apilimod having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers, in the form of lyophilized cake or aqueous solutions or mixtures. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic 20 acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; 25 sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as Tween, Pluronic, or polyethylene glycol (PEG).

 The term "buffer" as used herein denotes a pharmaceutically acceptable excipient, which stabilizes the pH of a pharmaceutical preparation. Suitable buffers are well known in the art and can be found in the literature. Pharmaceutically acceptable buffers include but 30 are not limited to histidine-buffers, citrate-buffers, succinate-buffers, acetate-buffers, phosphate-buffers, arginine-buffers or mixtures thereof. The above-mentioned buffers are generally used in an amount of about 1 mM to about 100 mM, of about 5 mM to about 50 mM and of about 10-20 mM. The pH of the buffered solution can be at least 4.0, at least

4.5, at least 5.0, at least 5.5 or at least 6.0. The pH of the buffered solution can be less than 7.5, less than 7.0, or less than 6.5. The pH of the buffered solution can be about 4.0 to about 7.5, about 5.5 to about 7.5, about 5.0 to about 6.5, and about 5.5 to about 6.5 with an acid or a base known in the art, e.g. hydrochloric acid, acetic acid, phosphoric acid, sulfuric acid and citric acid, sodium hydroxide and potassium hydroxide. As used herein when
5 describing pH, "about" means plus or minus 0.2 pH units.

Examples of pharmaceutically acceptable surfactants include polyoxyethylenesorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene
10 copolymer (Poloxamer, Pluronic), and sodium dodecyl sulphate (SDS). Suitable surfactants include polyoxyethylenesorbitan-fatty acid esters such as polysorbate 20 and polysorbate 80. When polysorbate 20 and polysorbate 80 are used they are generally used in a concentration range of about 0.001 to about 1%, of about 0.005 to about 0.2% and of about 0.01% to about 0.1% w/v (weight/volume).

15 As used herein, the term "stabilizer" can include a pharmaceutical acceptable excipient, which protects the active pharmaceutical ingredient and/or the formulation from chemical and/or physical degradation during manufacturing, storage and application. Stabilizers include but are not limited to sugars, amino acids, polyols, cyclodextrins, e.g. hydroxypropyl-beta-cyclodextrin, sulfobutylethyl-beta-cyclodextrin, beta-cyclodextrin,
20 polyethyleneglycols, e.g. PEG 3000, PEG 3350, PEG 4000, PEG 6000, albumin, human serum albumin (HSA), bovine serum albumin (BSA), salts, e.g. sodium chloride, magnesium chloride, calcium chloride, chelators, e.g. EDTA as hereafter defined. As mentioned hereinabove, stabilizers can be present in the formulation in an amount of about 10 to about 500 mM, an amount of about 10 to about 300 mM, or in an amount of about
25 100 mM to about 300 mM.

For oral administration, the compounds (i.e., apilimod and other therapeutic agents) can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries,
30 suspensions and the like, for oral ingestion by a subject to be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars,

including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers, i.e. EDTA for neutralizing internal acid conditions or may be administered without any carriers.

Also specifically contemplated are oral dosage forms of apilimod or other agents. Apilimod or other agents may be chemically modified or mixed with other components so that oral delivery is efficacious. Generally, the chemical modification or mixture contemplated permits (a) longer half-lives; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of apilimod or other agents and increase in circulation time in the body. Examples of such moieties or other compounds include: polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are polyethylene glycol moieties.

The location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine. Preferably, the release will avoid the deleterious effects of the stomach environment, either by protection of apilimod or other agent, or by release of the biologically active material beyond the stomach environment, such as in the intestine.

To ensure full gastric resistance a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

Tablets may be coated with a coating or mixture of coatings that is not intended for protection against the stomach. This can include sugar coatings, or coatings that make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic, i.e. powder; for liquid forms, a soft gelatin shell may be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

The therapeutic can be included in the formulation as fine multi-particulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colorants and flavoring agents may all be included. For example, apilimod may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

One may dilute or increase the volume of the therapeutic with an inert material. These diluents could include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrates include but are not limited to starch, including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An anti-frictional agent may be included in the formulation to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the therapeutic into an aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential non-ionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation either alone or as a mixture in different ratios.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

Kits

The invention also includes kits. The kit has a container housing apilimod and optionally additional containers with other therapeutics such as anti-viral agents or viral

vaccines. The kit also includes instructions for administering the component(s) to a subject who has or is at risk of having a viral infection as described herein.

In some aspects of the invention, the kit can include a pharmaceutical preparation vial, a pharmaceutical preparation diluent vial, and inhibitor. The vial containing the diluent
5 for the pharmaceutical preparation is optional. The diluent vial contains a diluent such as physiological saline for diluting what could be a concentrated solution or lyophilized powder of inhibitor. The instructions can include instructions for mixing a particular amount of the diluent with a particular amount of the concentrated pharmaceutical preparation, whereby a final formulation for injection or infusion is prepared. The
10 instructions may include instructions for use in an oral formulation, inhaler, intravenous injection, intraperitoneal injection, subcutaneous pump, or any other device useful according to the invention. The instructions can include instructions for treating a patient with an effective amount of apilimod. It also will be understood that the containers containing the preparations, whether the container is a bottle, a vial with a septum, an
15 ampoule with a septum, an infusion bag, a pump, and the like, can contain indicia such as conventional markings which change color when the preparation has been autoclaved or otherwise sterilized.

EXPERIMENTAL DETAILS

This invention will be better understood from the Experimental Details, which
20 follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

Example 1 – Activity of apilimod against cells pseudotyped with virus glycoproteins

Vero cells were treated with apilimod for one hour and then challenged with murine
25 retrovirus vectors encoding GFP and pseudotyped with the indicated virus glycoprotein - EboV (Zaire), Marburg (MARV), SARS, MERS, Lassa fever virus (LFV), or vesicular stomatitis virus G (VSV) - all of which are dependent on acid pH. SARS and MERS are known to require the same lysosomal proteases for infection that are utilized by EboV and Marburg virus. After four hours, virus and apilimod were removed and two days later, the
30 number of cells with acquired GFP was determined.

The findings are presented in Figure 1 as a function of the concentration of apilimod in the culture medium. The findings show that apilimod inhibits infection by EboV, Marb,

SARS and MERS GP, but not LFV or VSV-G glycoproteins. Apilimod is potent - IC₅₀ is in the low nanomolar range.

While not wishing to be bound by any particular theory, these data suggest that apilimod targets the viral trafficking pathway of cultured cells. Because EboV and Marburg are strictly dependent on lysosome NPC1, *in vivo* infection may be susceptible to apilimod. Mouse and primate models of EboV and Marburg infection are planned.

Example 2 – Activity of apilimod against cells pseudotyped with glycoproteins from various species of Filoviridae

Example 1 was repeated using MLV particles pseudotyped with GPs from EboV Zaire (ZEboV), Sudan (SEboV), Cote d'Ivoire (CIEboV), Reston (REboV), and Bundibugyo (BEboV), Marburg (MarV) and Lassa fever (LFV) viruses, and found that apilimod is broadly active against infection mediated by GPs from these various species of *Filoviridae*. See Figure 2.

Example 3 – Comparison of activity of apilimod to activity of other anti-virals in cells pseudotyped with glycoproteins from various viruses

Other viruses are known to require endocytosis and acid pH. A larger set of MLV particles was pseudotyped with glycoproteins from these viruses and examined by the assay described in Example 1. Anti-viral compounds investigated were chloroquine (CQ) (a known antiviral compound), Compound 10 (a known EboV inhibitor), and apilimod.

Inhibition of EboV, Marburg, and SARS was confirmed for both Compound 10 and apilimod. In addition, these compounds did not target infection by other acid pH, endocytosis-dependent viruses that do not use phagosome-lysosome trafficking pathways (including Lassa (LFV), VSV G, Nipah virus (NiV), lymphochoriomeningitis virus (LCMV), murine retrovirus Env (MLV), influenza HA (Flu), and arenavirus Junin (not shown)). See Figure 3. This overlap in anti-viral profile of apilimod and Compound 10 provides additional evidence that these compounds target a specific virus entry pathway that is used by EboV and Marburg viruses.

Example 4 – Comparison of activity of apilimod to activity of Compound 10 in cells pseudotyped with glycoproteins from mouse-adapted EboV

In anticipation of studies in mice, the effect of apilimod and Compound 10 on infection by MLV pseudotyped with GP from a mouse-adapted strain of EboV was examined. The mouse-adapted strain of EboV is the strain that will be used in studies of mice. Infectivity was examined in Vero cells (Figure 4A) and also in primary mouse

fibroblast cells (Figure 4B). Apilimod is a less potent inhibitor of EboV GP infection in mouse cells than in Vero cells.

Example 5 – Activity of apilimod against cells infected with Ebolavirus or Marburg virus

The effect of apilimod on growth of *bona fide* Ebolavirus and Marburg viruses in Vero cells was examined in collaboration with researchers at the BL4 facility USAMRIID at Ft. Detrick, MD. Cells were treated with apilimod at the concentrations indicated in Figure 5 for one hour and then challenged with Ebola virus Zaire (strain engineered to encode GFP) or Marburg virus Angola strain (moi=0.01). Virus growth was monitored by enumerating GFP positive cells and by EboV Zaire GFP and by qPCR for Marburg virus after 72 hours using a well-characterized assay.

Virus titer increased by greater than 1000-fold in cells treated with DMSO vehicle. However, apilimod inhibited growth of both viruses. The IC₅₀ for apilimod is 10-12 nM. An additional control indicator for ATP content (CC₅₀) showed that apilimod is not cytotoxic.

Example 6 - Assay to localize action of EboV inhibitors

Cultured RAW264.7 macrophage cells were incubated with dextran particles that are the same size as virus particles and are labeled with rhodamine (red). These cells were chosen because phagocytosis is robust. After one hour, the particles were removed and cells were cultured for additional six hours to allow red-dextran particles taken up by phagocytosis to reach the lysosome. The red-dextran particles are inert, so they remain in place.

Cells were then treated with an EboV inhibitor or DMSO control and challenged with green dextran particles for one hour. Dextran were removed but inhibitors were continued for six hours. See Figure 6A. Cells and dextrans were then visualized by microscopy and scored for co-localization of red and green dextrans in cytoplasmic vesicles - presumed lysosomes.

Strong co-localization (red+green = yellow) was found in cells treated with DMSO or with a known NPC1 inhibitor. However, yellow spots were markedly reduced (>80%) in cells treated with Compound 10. This indicates that this compound interferes with vesicle trafficking to lysosomes. See Figure 6B, arrows indicate yellow spots.

While not wishing to be bound by any particular theory, it is hypothesized that apilimod functions in a similar manner as Compound 10, and that the assay described above will produce similar results for apilimod.

Assay reference: Harrison R.E., et al. Laboratory of Sergio Grinstein, Toronto. *Molecular and Cellular Biology* 2003, 23, 6494-506.

Example 7 - Correlation between the anti-EboV activity of a series of EboV inhibitors and their activity in blocking lysosomal degradation marker protein LC3-II

5 Human HT1080 cells were treated with Compound 10 or a structural analog thereof for four hours, then lysed. The post-nuclear lysate was analyzed by SDS-PAGE and immunoblot using anti-LC3 antibody for the presence of the LC3-II. LC3-II is a marker for autophagosomes, which are transporter to lysosomes where LC3-II is degraded. The findings show a strong correlation between capacity of Compound 10, Compound 5, and
10 Compound 2 to inhibit infection and to inhibit degradation of LC3-II. See Figure 7.

Human HT1080 cells were treated with apilimod at 50 nM and 100 nM concentrations. Apilimod treatment increased steady state level of lysosome trafficking marker LC3-II. See Figure 8.

Also, additional experiments were performed (data not shown) that clearly show
15 that neither EboV infection nor Compound 10 stimulates autophagy or production of LC3-II. These findings are consistent with results of rhodamine trafficking studies (Example 6, above) to indicate that Compound 10 blocks vesicle trafficking to lysosomes.

Example 8 – Identification of target of EboV inhibitors

Compound 99 was synthesized. It is a structural analog of Compound 10 that
20 contains both an aryl azide moiety suitable for photo-reactive covalent cross-linking and an alkyne moiety that is a substrate for Cu catalyzed coupling to epitope AlexaFluor 488. Compound 99 retains anti-EboV activity ($IC_{50} = 0.75 \mu M$).

Figure 9A depicts a schematic representation of a protocol for using Compound 99 for target identification.

25 Compound 99 (10 micromolar) was incubated with human HT1080 cells. The cells exposed to UV light for 5 minutes. Cells were lysed and post-nuclear membranes were incubated with Cu catalyst in the presence of AlexaFluor488 azide. Targeted proteins were immunoprecipitated with anti-Alexa-Fluor 488 antibody.

The candidate protein (indicated by the black arrow in Figure 9B) corresponds in
30 size to Vac14.

Example 9 – Identification of PIP/HOPS proteins as targets for EboV inhibitors

Vac14 was expressed with human influenza hemagglutinin (HA) epitope tag, immunoprecipitated with HA-directed antibody, and analyzed for Compound 99 using anti-

AlexaFluor 488 antibody. *See* Figure 10. This experiment indicates Vac14 is a target for Compound 99 in cells. Other proteins in PIP and in HOPS complexes were not labeled with Compound 99.

Example 10 – Role of PIP complex in EboV infection

5 As additional test of the role of PIP complex in EboV infection, an HT1080 cell line was created in which the Fig4 subunit of the PIP complex was deleted. These cells were challenged with MLV pseudotyped particles. The same pattern same pattern of infection was observed as described above (i.e., specific inhibition of EboV and SARS GP-dependent infection). *See* Figure 11.

10

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

15

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The appended claims are not intended to claim all such embodiments and variations, and the full scope of the invention should be determined by reference to the claims, along with their full scope of
20 equivalents, and the specification, along with such variations.

What is claimed is:

1. A method of treating or preventing a viral infection in a subject in need thereof comprising administering to the subject an effective amount of apilimod, or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer, or stereoisomer thereof, wherein the viral infection is caused by a virus classified in of the genera *Ebolavirus* or *Marburgvirus*.
2. The method of claim 1, wherein the subject is a subject having a viral infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to treat the viral infection.
3. The method of claim 1, wherein the subject is a subject at risk of having a viral infection caused by a virus classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to prevent viral infection of the subject.
4. The method of any one of claims 1-3, wherein the virus is *Zaire ebolavirus*, *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*.
5. The method of any one of claims 1-4, wherein the virus is *Zaire ebolavirus*.
6. The method of any one of claims 1-4, wherein the virus is *Marburg marburgvirus*.
7. The method of any one of claims 1-6, wherein the subject is human.
8. The method of any one of claims 1-7, wherein apilimod is administered intravenously, intraperitoneally, or subcutaneously.
9. The method of any one of claims 1-8, wherein apilimod is administered continuously.
10. The method of claim 9, wherein apilimod is administered continuously using a pump device.
11. A method of inhibiting PIKfyve in a cell that has been infected with or is at risk of being infected with a virus classified in the genera *Ebolavirus* or *Marburgvirus* comprising contacting the cell with an effective amount of apilimod, or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer, or stereoisomer thereof.
12. The method of claim 11, wherein the cell has been infected with a virus classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to treat the infection of the cell.

13. The method of claim 11, wherein the cell is at risk of being infected with a virus classified in the genera *Ebolavirus* or *Marburgvirus*; and the effective amount of apilimod is an amount effective to prevent infection of the cell.
14. The method of any one of claims 11-13, wherein the virus is *Zaire ebolavirus*,
5 *Marburg marburgvirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, *Sudan ebolavirus*, or *Tai Forest ebolavirus*.
15. The method of any one of claims 11-14, wherein the virus is *Zaire ebolavirus*.
16. The method of any one of claims 11-14, wherein the virus is *Marburg marburgvirus*.
- 10 17. The method of any one of claims 11-16, wherein the cell is a human cell.
18. The method of any one of claims 11-17, wherein the cell is in vitro.
19. The method of claim 18, wherein the IC₅₀ of apilimod is less than about 50 nM, less than about 40 nM, less than about 30 nM, or less than about 20 nM.
20. The method of any one of claims 11-17, wherein the cell is in vivo.
- 15 21. The method of any one of claims 11-20, wherein the cell is a liver cell.
22. The method of any one of claims 11-21, wherein the cell is a hepatocyte.

Figure 1

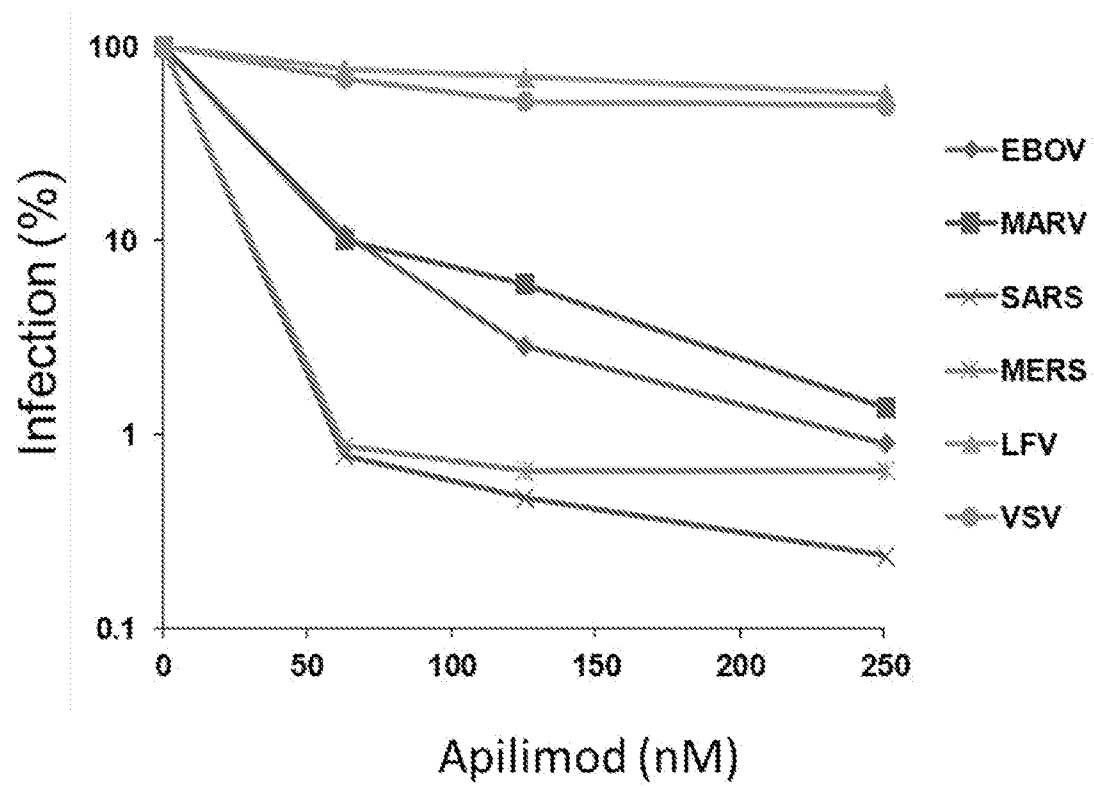


Figure 2

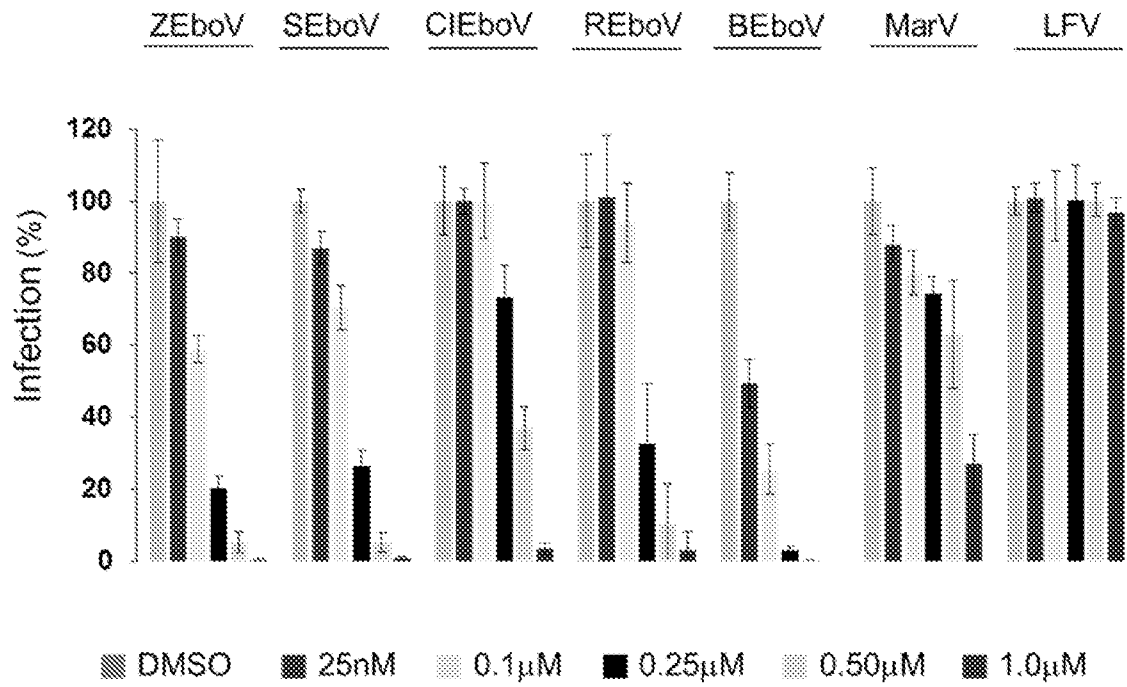


Figure 3

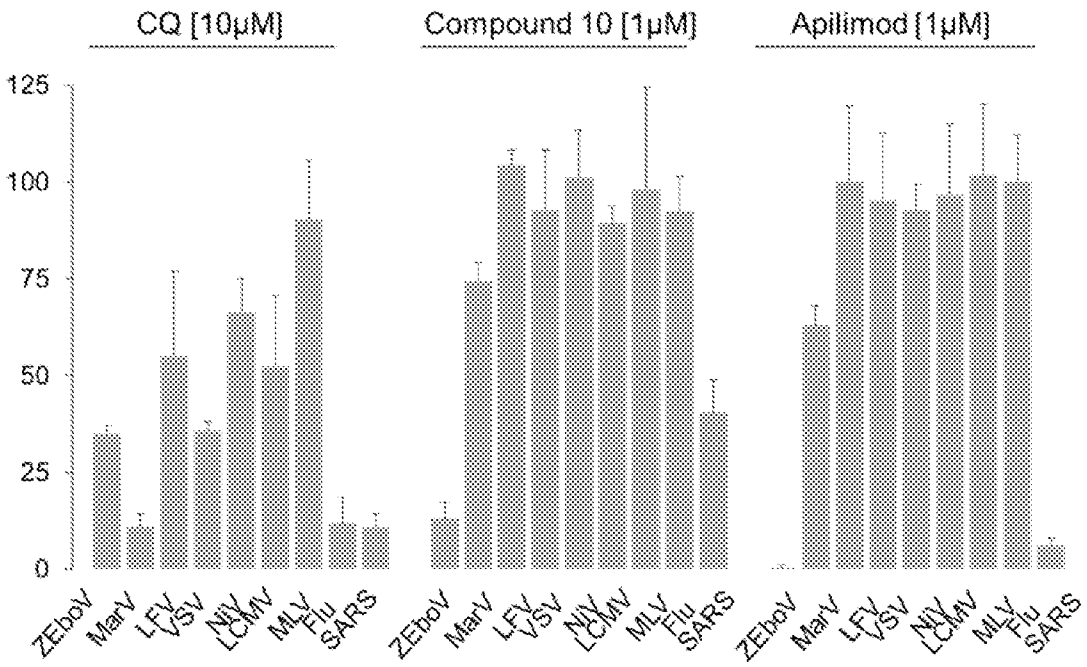


Figure 4A

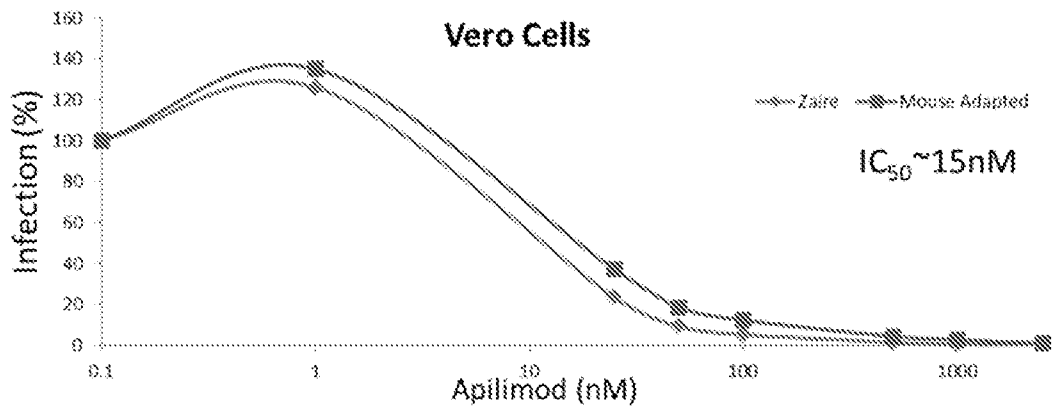


Figure 4B

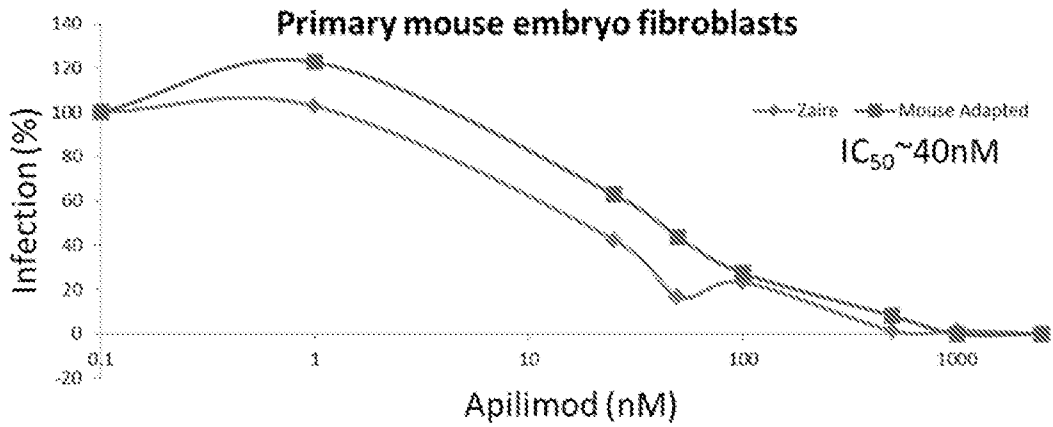


Figure 5

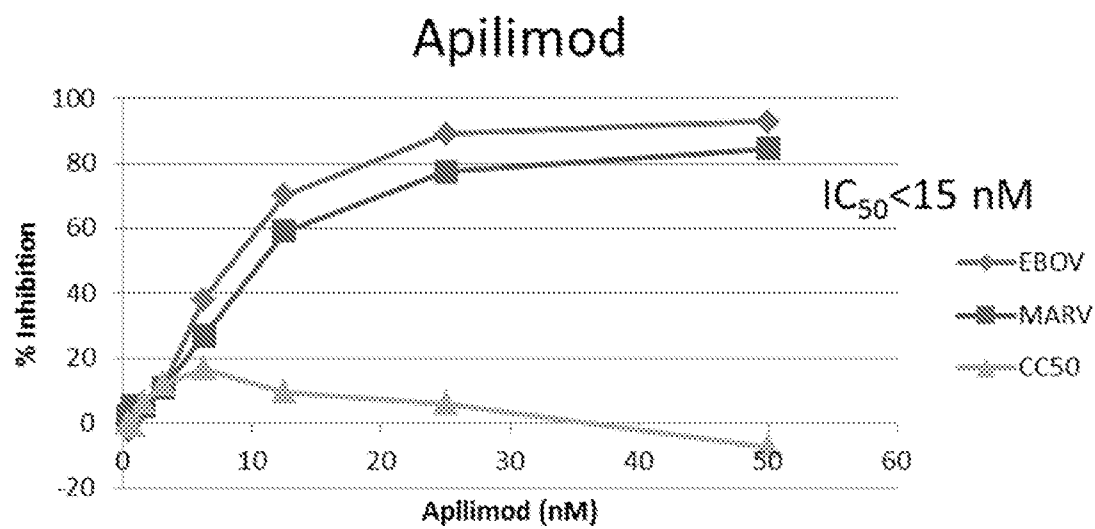


Figure 6A



Figure 6B

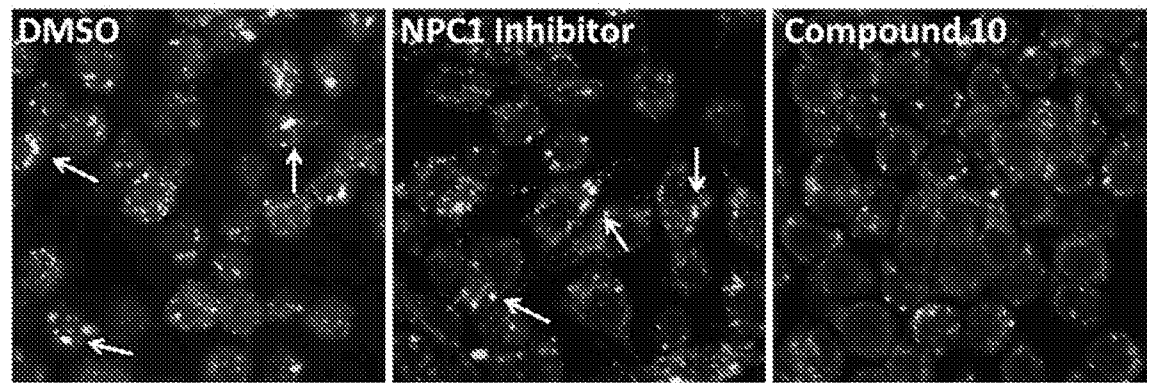


Figure 7

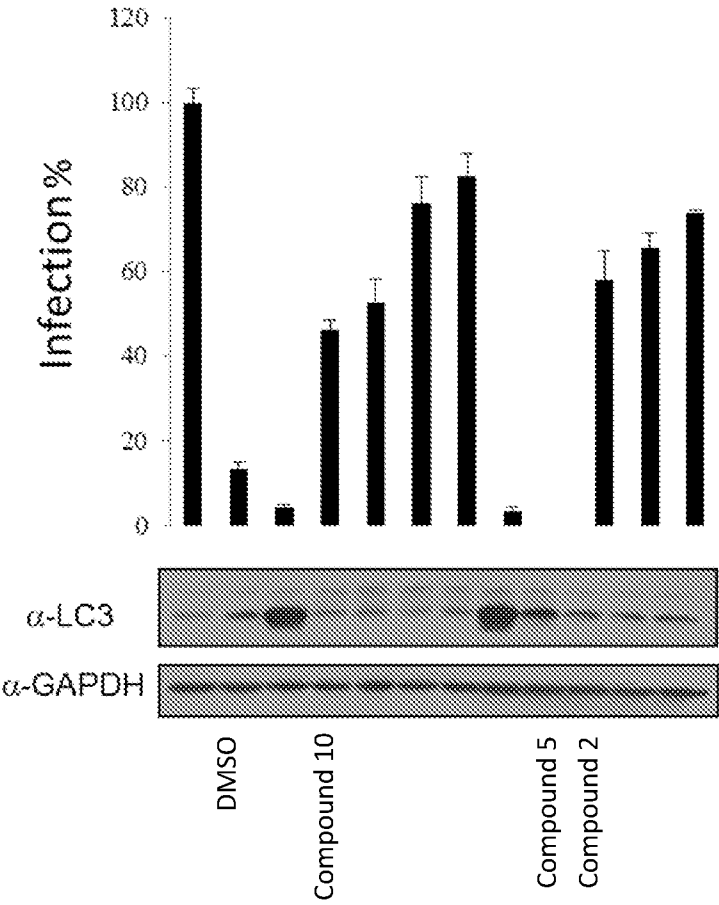


Figure 8

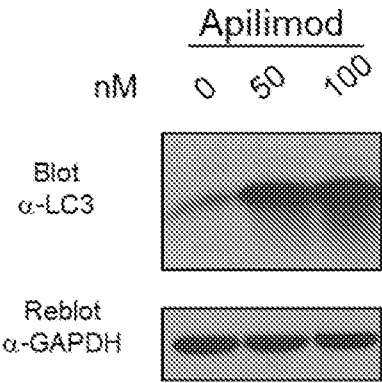


Figure 9A

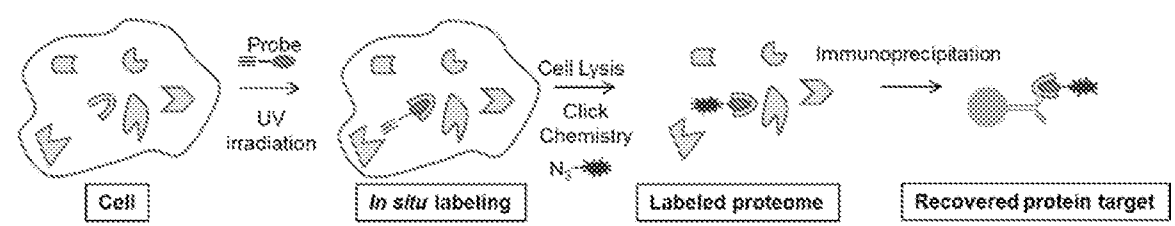


Figure 9B

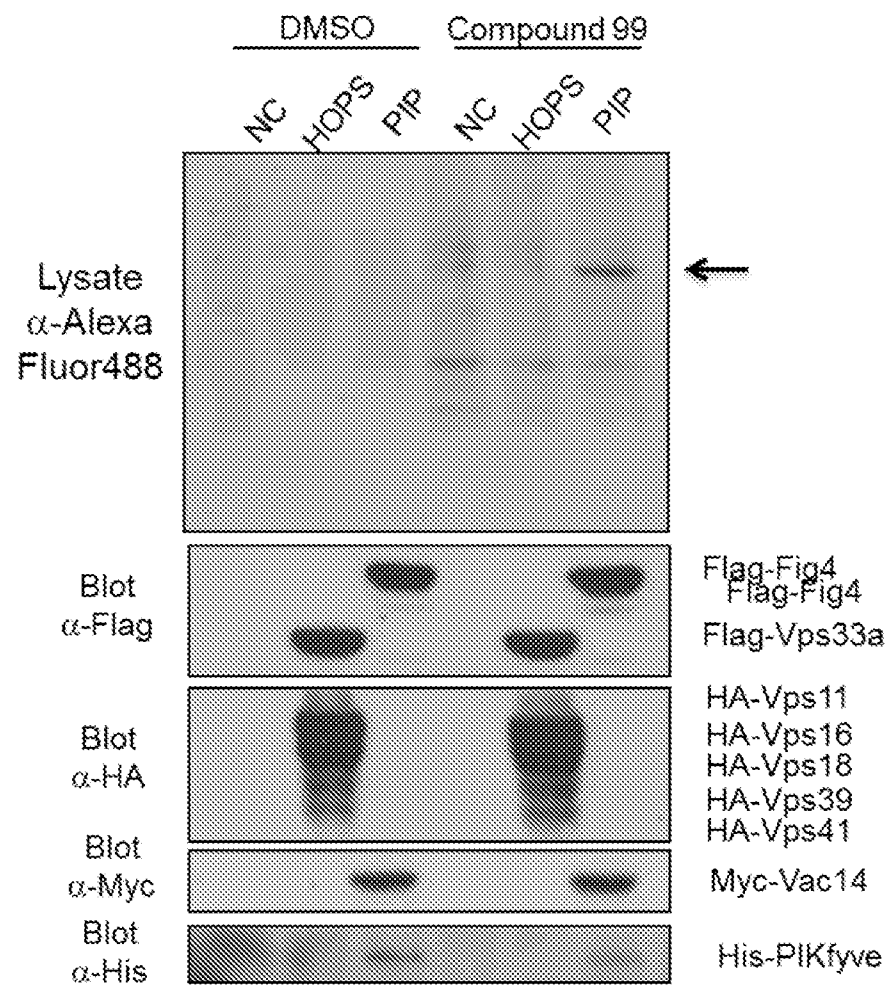


Figure 10

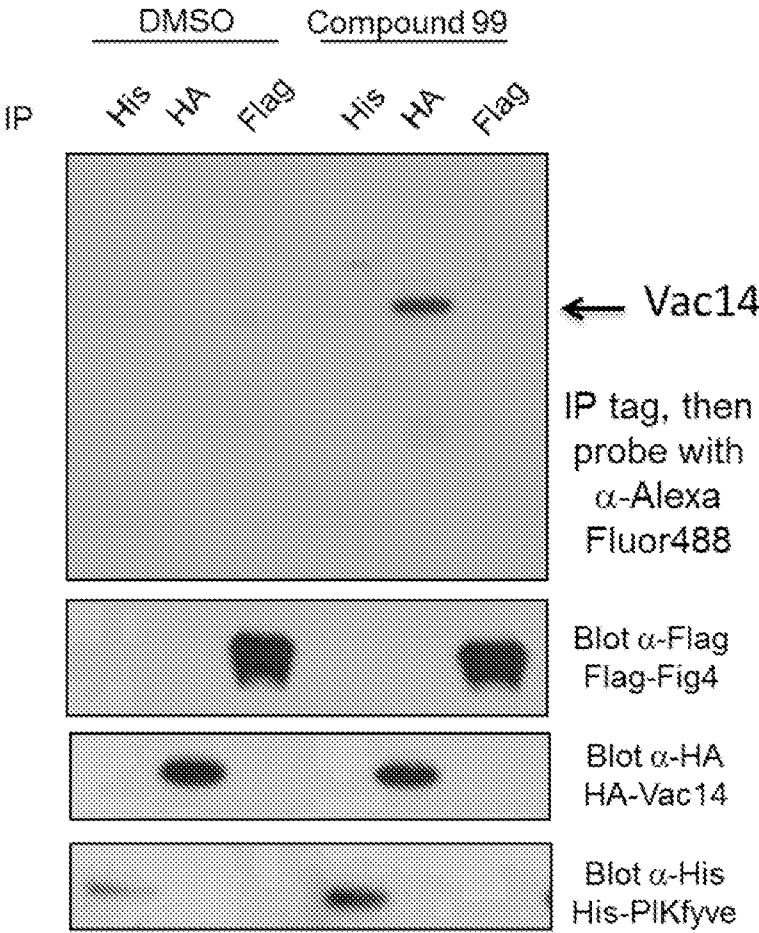
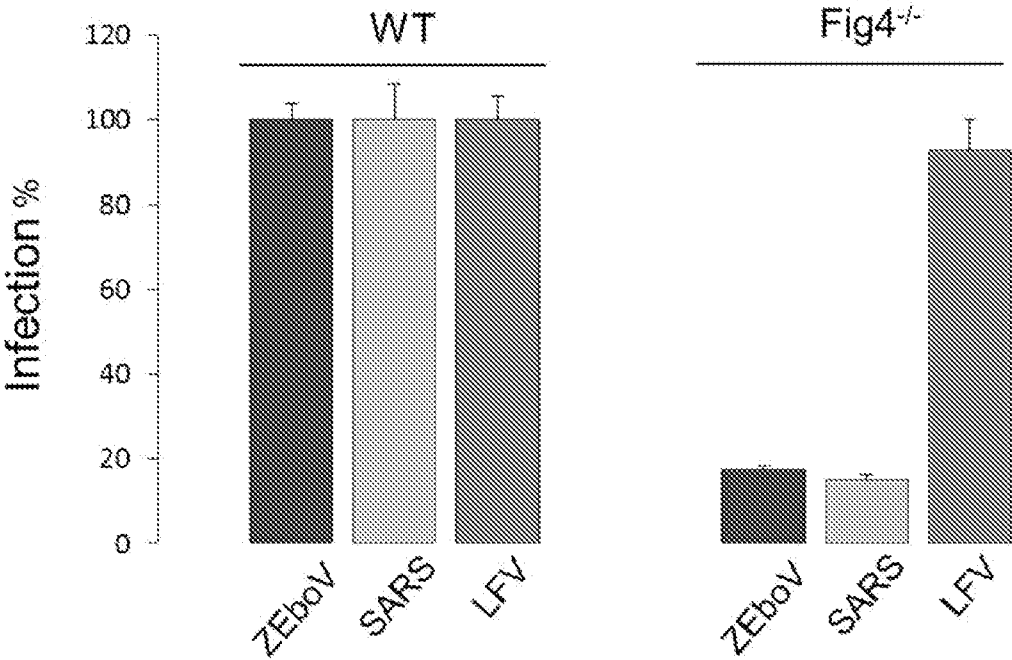


Figure 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/12284

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 31/5377; A61K 31/506; A61P 31/12 (2016.01) CPC - C07D239/02; C07D413/14; A61K31/5377; A61K31/506 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) IPC(8) - A61K 31/5377; A61K 31/506; A61P 31/12 (2016.01) CPC - C07D239/02; C07D413/14; A61K31/5377; A61K31/506 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 514/231.5; 514/256; 514/639 (keyword limited; terms below) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, Google Scholar, Search Terms: apilimod, PIKfyve inhibitor, viral infection, ebolavirus/Marburgvirus, treat, prevent/prophylaxis		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0018338 A1 (CHANDRAN et al.) 16 January 2014 (16.01.2014) entire document, especially Abstract and para [0120]	1-4, 11-14
Y	US 2015/0004133 A1 (KISSELEVA et al.) 01 January 2015 (01.01.2015) entire document, especially Abstract, paras [0042]; [0056]; [0058]	1-4, 11-14
Y	US 2014/0329834 A1 (CUNNINGHAM et al.) 06 November 2014 (06.11.2014) entire document, especially para [0036]	4, 14
☐ Further documents are listed in the continuation of Box C. ☐		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 21 February 2016 (21.02.2016)		Date of mailing of the international search report 17 MAR 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/12284

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.: 5-10 and 15-22
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.