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## SIRNA SILENCING OF FILOVIRUS GENE EXPRESSION

### CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/729,476, filed Oct. 20, 2005, the disclosure of which is hereby incorporated by reference in its entirety for all purposes.

### REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK

The Sequence Listing written in file -56-1.APP, 7,880,704 bytes, machine format IBM-PC, MS-Windows operating system, created on Feb. 5, 2007 on duplicate copies of compact disc of the written form of the Sequence Listing, i.e., "Copy 1 of 3" and "Copy 2 of 3", and the sequence information recorded in computer readable form on compact disc, i.e., "Copy 3 of 3" for application Ser. No: 11/584,341, MacLachlan et al., SIRNA SILENCING OF FILOVIRUS GENE EXPRESSION, is hereby incorporated by reference.

### BACKGROUND OF THE INVENTION

Filoviruses (e.g., Ebola virus and Marburg virus) are among the most lethal and destructive viruses. They cause severe, often-fatal viral hemorrhagic fevers in humans and nonhuman primates (e.g., monkeys, gorillas, and chimpanzees). Filoviruses are of particular concern as possible biological weapons since they have the potential for aerosol dissemination and weaponization.

The incubation period for Filovirus infection ranges from 2 to 21 days. The onset of illness is abrupt and is characterized by high fever, headaches, joint and muscle aches, sore throat, fatigue, diarrhea, vomiting, and stomach pain. A rash, red eyes, hiccups and internal and external bleeding may be seen in some patients. Within one week of becoming infected with the virus, most patients experience chest pains and multiple organ failure, go into shock, and die. Some patients also experience blindness and extensive bleeding before dying.

Filoviridae are a family of RNA viruses. Two members of the Filoviridae family have been identified: Ebola virus and Marburg virus. There is one identified strain of Marburg virus and four identified subtypes (i.e., strains) of Ebola virus: Ebola-Zaire, Ebola-Sudan, Ebola-Ivory Coast (i.e., Ebola-Tai), and Ebola-Reston. The exact origin, locations, and natural habitat of Filoviridae unknown. However, on the basis of available evidence and the nature of similar viruses, it is postulated that Filoviridae are zoonotic (i.e., animal-borne) and are normally maintained in an animal host that is native to the African continent.

Because the natural reservoir of the virus is unknown, the manner in which the virus first appears in a human at the start of an outbreak has not been determined. It is hypothesized that the first patient becomes infected through contact with an infected animal. After the first case-patient in an outbreak setting is infected, the virus can be transmitted in several ways. People can be exposed to the virus from direct contact with the blood and/or secretions of an infected person. Thus, the virus is often spread through families and friends because they come in close contact with such secretions when caring for infected persons. People can also be exposed to the virus through contact with objects contaminated with infected

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secretions (e.g., needles or syringes). All Filoviridae species have also displayed the ability to be spread through airborne particles (i.e., via aerosol).

Prevention and treatment for Filovirus infection presents many challenges. Because the identity and location of the natural reservoir of the viruses are unknown, there are few effective preventative measures. There is currently no treatment for Filovirus infection. Patients receive supportive therapy, i.e., electrolyte and fluid balancing, oxygen, blood pressure maintenance, and treatment for any secondary infections.

Thus, there is a need for compositions and methods for treating and preventing filovirus infection, e.g., by specifically modulating filovirus gene expression. The present invention addresses these and other needs.

### SUMMARY OF THE INVENTION

The present invention provides siRNA molecules that target Filovirus gene (e.g., L-pol, VP24, VP30, VP35, VP40, nucleoprotein (NP), and/or glycoprotein (GP)) expression and methods of using such siRNA molecules to silence Filovirus (e.g., Ebola virus or Marburg virus) gene expression.

In one aspect, the present invention provides an siRNA molecule comprising a double-stranded sequence of about 15 to about 60 nucleotides in length (e.g., about 15-60, 15-50, 15-40, 15-30, 15-25, or 19-25 nucleotides in length), wherein the siRNA molecule silences expression of a Filovirus gene such as L-pol, VP24, VP30, VP35, VP40, NP, and/or GP from Ebola virus or Marburg virus. In certain instances, the double-stranded sequence comprises a hairpin loop structure.

In some embodiments, the siRNA molecule has 3' overhangs of one, two, three, four, or more nucleotides on one or both sides of the double-stranded region. In other embodiments, the siRNA molecule lacks overhangs (i.e., have blunt ends). Preferably, the siRNA molecule has 3' overhangs of two nucleotides on each side of the double-stranded region. Examples of 3' overhangs include, but are not limited to, 3' deoxythymidine (dT) overhangs of one, two, three, four, or more nucleotides.

In certain instances, the siRNA molecule comprises at least one modified nucleotide in the sense and/or antisense of the sequence. As a non-limiting example, the siRNA molecule can be selectively modified at less than about 20% of the nucleotides in the sequence. Preferably, the modified siRNA contains at least one 2'OMe purine or pyrimidine nucleotide such as a 2'OMe-guanosine, 2'OMe-uridine, 2'OMe-adenosine, and/or 2'OMe-cytosine nucleotide. The modified siRNA molecule is notably less immunostimulatory than a corresponding unmodified siRNA sequence and is capable of silencing expression of the target Filovirus gene.

The siRNA molecule may comprise at least one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences set forth in Tables 1-15. In some embodiments, the siRNA molecule comprises at least one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences set forth in Tables 1-13. Preferably, the siRNA molecule comprises at least one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences set forth in Tables 1-2, such as, e.g., EK1, EK2, EK3, and/or EK4.

In some embodiments, the siRNA molecule described herein further comprises a carrier system, e.g., to deliver the siRNA molecule into a cell of a mammal. Non-limiting examples of carrier systems suitable for use in the present invention include nucleic acid-lipid particles, liposomes, micelles, virosomes, nucleic acid complexes, and mixtures