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# EMERGING THERAPEUTIC OPTIONS AGAINST FILOVIRUSES

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## SUMMARY

*The highly pathogenic ebolaviruses and marburgviruses remain a significant human health concern since their discovery over 40 years ago. Sporadic outbreaks resulting in high fatality rates and the potential use of these viruses as bioweapons have prompted intense investigations aimed at identifying therapeutic countermeasures. While recent emphasis has been placed on the development of filovirus vaccine candidates, in particular, the use of replicating or non-replicating vaccine platforms, there has been significant progress made towards the discovery and development of small-molecule and nucleic acid-based therapeutics. A number of these advances have resulted from the identification of new viral or host cell targets. These targets are found through continued investigation and elucidation of the mechanisms underlying viral pathogenesis. In this review, we discuss the progress of these novel small-molecule inhibitors, as well as recent advances in gene targeting against filoviruses. In addition, emerging data on cellular defense systems, such as the antioxidant response, will be addressed.*

**Key words:** Filoviruses – Ebolavirus – Marburgvirus – Small-molecule inhibitors – Antioxidants

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## INTRODUCTION

Ebolaviruses and marburgviruses cause severe hemorrhagic disease in humans and are widely regarded as among the most lethal pathogens known. These viruses cause sporadic outbreaks and are endemic to regions in sub-Saharan Africa; additionally, recent reports indicate that ebolaviruses and ebola-like viruses may also be endemic to the Philippines and Europe as well (1, 2).

Ebolaviruses and marburgviruses comprise the *Filoviridae* family. Currently, there are five known species of ebolaviruses: Ebola virus (EBOV; previously referred to as Zaire), Sudan virus (SUDV), Reston virus (RESTV), Taï Forest virus (TAFV; previously called Côte d'Ivoire), and the most recently discovered member, Bundibugyo virus (BDBV) (3). There is a single marburgvirus species with two members: Marburg virus (MARV) and Ravn virus (RAVV). A third lineage of filoviruses has recently been proposed as a genus, "Cuevavirus", with a single species, "Lloviu cuevavirus", following sequence identification of a novel Lloviu virus (LLOV) (2, 4). To date, LLOV RNA has only been identified in insectivorous bats (2), and its pathogenicity in humans is unknown.

Filoviruses are filamentous, nonsegmented, negative-sense, single-strand RNA viruses with a genome approximately 19 kb in length. The genome encodes seven structural proteins: the nucleoprotein (NP), matrix protein VP40, polymerase cofactor VP35, envelope glycoprotein ($GP_{1,2}$), minor nucleoprotein VP30, membrane-associated protein VP24 and the RNA-dependent RNA polymerase L (L). The viral ribonucleoprotein complex consists of NP, VP35, VP30, L and VP24 (5, 6). The matrix protein VP40 serves as the major matrix protein, while $GP_{1,2}$ mediates cell entry.

Filoviruses are classified as NIAID Category A Priority Pathogens because of the high case fatality rates observed in humans during outbreaks (up to 90%), the possibility to aerosolize the viruses and concerns about the viruses being used as bioweapons (7, 8). In addition, there are currently no FDA-approved antiviral therapeutics or vaccines available for treating these viruses. Altogether, these attributes have fueled research in private, academic and government laboratories aimed at identifying effective vaccine and therapeutic candidates. From these studies, several promising candidate vaccines and therapeutics have emerged and are currently in various stages

of development. This review will focus on recent therapeutic advances in the field; specifically, we will focus on recent advancements using small interfering RNA (siRNA), antisense oligonucleotide and small molecules, such as antioxidants. We will also discuss insight gained from recent studies on viral pathogenesis and the implications these studies have for the design of future filovirus therapeutics.

## FILOVIRUS MOLECULAR BIOLOGY

In addition to roles in the viral life cycle, several proteins encoded by filoviruses have been demonstrated to have other functions. The EBOV VP35 protein, in addition to being an integral member of the replication/transcription complex, also functions as an antagonist of the type I interferon (IFN) system (9). Recent evidence indicates that EBOV VP35 inhibits the type I IFN system in multiple ways (10-12). EBOV VP35 inhibits IFN-beta promoter activation mediated by the retinoic acid-inducible gene I (RIG-I) protein signaling pathway, at least in part by an interaction with the interferon regulatory factor 3/7 (IRF-3/7) kinases TANK binding kinase 1 (TBK1) and inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKK-E), where it serves as an alternate substrate (12). In addition, EBOV VP35 was found to interact with IRF-7, SUMO-conjugating enzyme UBC9 and E3 SUMO-protein ligase PIAS1 (11). In this study, VP35 was found to promote SUMOylation of IRF-3 and IRF-7, a modification that resulted in impairment of IFN-beta promoter activity. It will be of interest to delineate the contribution of these two functions to the inhibition of IRF-3 and IRF-7 during EBOV infection. EBOV VP35 also inhibits the activation of the dsRNA-activated protein kinase (PKR) (13). PKR is a double-stranded RNA (dsRNA) activated serine/threonine-protein kinase which is known to inhibit virus replication by blocking translation via phosphorylation of eIF-2alpha (14). EBOV VP35 can also suppress RNA silencing, a function that appears to be dependent on the RNA binding activity of VP35 (15, 16). Interestingly, this function may be shared by other EBOV proteins. A recent report identified EBOV VP40 and VP30 as suppressors of RNA silencing, highlighting the multifunctional nature of these proteins as well (15).

EBOV VP24, similar to VP35, has also been demonstrated to antagonize the host IFN system (17). EBOV VP24 was found to block nuclear translocation of tyrosine-phosphorylated signal transducer and activator of transcription (STAT1) through an interaction with the nucleoprotein interactor 1 (NPI-1) family of karyopherins (17-19). The ability of EBOV VP24 to bind the NPI-1 family of karyopherins suggests that additional proteins that require these shuttling factors for nuclear import are likely to be affected. Indeed, a recent report identified the heterogeneous nuclear ribonuclear protein complex C1/C2 (hnRNP C1/C2) as a novel NPI-1 cargo protein that was impaired in nuclear shuttling in the presence EBOV VP24 (20). As more proteins are found to be dependent on the NPI-1 family of karyopherins for shuttling, a more global role for EBOV VP24 in regulating cytoplasmic nuclear transport of host cell proteins will likely emerge.

Although the mechanism(s) remains unclear, a role for EBOV NP and VP24 as critical virulence factors required for host adaptation has been indicated (21-24). Further studies will be needed to identify the particular function(s) of NP and VP24 that are required for adaptation. Of note, it does not appear that the IFN antagonist function of VP24 is required. A study by Mateo et al. showed that EBOV adaptation in guinea pigs is not associated with the IFN antagonist function of VP24 (24). It will be of interest to determine if the ability of VP24 to disrupt cytoplasmic nuclear shuttling is linked to adaptation.

Marburgviruses, in contrast to ebolaviruses, utilize the VP40 protein for antagonism of the host IFN system (25, 26). VP40 was demonstrated to inhibit the IL-6, type I and II IFN signaling pathways by inhibiting phosphorylation of Janus kinase 1 (JAK1) (25). The presence of JAK1 immediately downstream of a number of signaling receptors (27) suggests that VP40 will impact these pathways as well. Interestingly, whereas the IFN antagonist function of VP24 is not associated with adaptation, preliminary evidence indicates that for MARV, adaptation is linked, at least in part, to VP40 antagonism of the host IFN signaling (26).

In addition to mediating cellular entry, the filovirus $GP_{1,2}$ has been demonstrated to have a number of other functions, including impairing the cellular antiviral response (28). The *GP* gene of MARV encodes a single open reading frame and produces a full-length transmembrane $GP_{1,2}$. In contrast, the primary gene product of the EBOV *GP* gene is a secreted non-structural soluble GP (sGP) (29). Proteolytic cleavage of sGP during processing results in production of a soluble Δ-peptide, which is also secreted from cells (30). Transcriptional editing of EBOV GP mRNA results in a frame shift connecting two partially overlapping open reading frames, which causes production of the transmembrane $GP_{1,2}$. Membrane-bound $GP_{1,2}$ is also shed from the surface of infected cells by the cellular sheddase TNF-alpha-converting enzyme (TACE) (31). A third *GP* gene product, a small soluble GP (ssGP), was recently identified in EBOV-infected cells (32). Release of soluble forms of GP has long been thought to play an immunoregulatory role during infection. This was confirmed by in vitro studies showing that sGP protected endothelial cells from inflammatory cytokine-induced damage, proposing an antiinflammatory role for sGP (33). Additionally, the Δ-peptide has been shown to inhibit viral cell entry, indicating that this peptide may also play an important role in pathogenesis (34).

Intracellular $GP_{1,2}$ has been reported to induce cell cytotoxicity and cell surface protein downregulation (35-38), although this may depend on $GP_{1,2}$ levels, as moderate levels do not appear to induce these processes (39). Mechanistically, $GP_{1,2}$ interacts with the GTPase dynamin, thereby perturbing cell trafficking, in particular, of cell surface proteins which play key roles in cell attachment. This will likely result in the observed downregulation and cytotoxicity functions ascribed to $GP_{1,2}$ (37). A more recent study demonstrated that EBOV GP can mask the epitopes of cell surface proteins, and in this way gives the illusion of downregulation, adding a novel way in which $GP_{1,2}$ regulates cell surface proteins (40). Further studies will be required to determine the contribution of epitope masking and cell surface downregulation to GP-induced cytotoxicity during filovirus infection.

## FILOVIRUS PATHOGENESIS

In the current model of filovirus infection in humans, viral propagation occurs after the virus enters through skin lesions and/or mucous membranes (7, 41). Dendritic cells (DCs) and monocytes/macrophages are believed to be early targets of infection (42). In vivo evidence of this was shown by serial sacrifice studies in non-human pri-

mates (NHPs). In these studies, EBOV-infected DCs and macrophages were identified as early targets of infection, detected as early as 2 days post-infection (42, 43). It is hypothesized that early infection of these circulating monocyte-derived cells results in viral dissemination to peripheral lymphatic organs and subsequent viral spread to organs such as the liver. Additionally, early infection of these cells will directly impact orchestration of the immune response to infection. To the latter point, filovirus infection of DCs reportedly impairs maturation and cytokine release (44). Recent studies indicate, however, that this may depend on the DC subset in question. It was reported that while conventional DCs (cDCs) are productively infected with EBOV, plasmacytoid DCs (pDCs) are not, and moreover, do not produce IFN-alpha upon infection (45). The inability of EBOV to infect pDCs, known to produce copious amounts of type I IFN upon stimulation (46), perhaps serves as an additional mechanism of immune evasion. This is supported by a recent study in which EBOV VP35 was not able to inhibit IFN production by pDCs when expressed from a recombinant Newcastle disease virus (NDV) (47). In contrast to cDCs, infection of macrophages with EBOV results in activation and proinflammatory cytokine secretion (48, 49). T-lymphocyte bystander apoptosis is another pathogenic feature observed in humans and NHPs during the course of EBOV infection, although the overall importance for EBOV disease progression is unclear (50-52). Ultimately, these and other responses to infection lead to the hallmarks of fatal filovirus infection, namely, vascular dysfunction, disseminated intravascular coagulation (DIC) and subsequent hypovolemic shock (53, 54).

## GENE TARGETING-BASED THERAPEUTICS

In recent years, gene targeting approaches have emerged as a promising therapeutic option against filoviruses. These studies, summarized in Table I, use either RNA interference (RNAi) or phosphorodiamidate morpholino oligomers (PMOs).

RNAi represents a promising tool for the treatment of infectious diseases and many other conditions (55). It has been successfully applied to inhibit a wide range of viruses and several studies have highlighted the use of RNAi as a potential therapeutic (56, 57). RNAi-based therapy has been successfully used to treat experimental EBOV infection of NHPs (58). In these studies, to improve the serum half-life of siRNAs in vivo, siRNAs were encapsulated in specialized liposomes to form stable nucleic acid lipid particles (SNALPs). SNALP-encapsulated siRNAs against the EBOV *L* gene administered intraperitoneally (i.p.) at 1.0 mg/kg protected three of five guinea pigs from lethal infection with a guinea pig-adapted EBOV, while all control animals succumbed to infection. These data indicate that SNALP-encapsulated L siRNA offered significant protection against viral disease. Moreover, of the two treated guinea pigs that succumbed, one died on day 6 with no detectable viremia, suggesting that viral replication was suppressed and death was likely due to therapy-dependent toxicity. The second was euthanized on day 26 and also appeared to be aviremic. This prompted a second evaluation with a lower dose of SNALP L siRNAs (0.75 mg/kg); here, all treated animals were protected from lethal virus challenge. In a follow-up study using NHPs and a combination of SNALP L, VP24 and VP35 siRNAs, given by intravenous (i.v.) infusion at 30 minutes, 1, 3 and 5 days post-challenge, two of three animals were protected from lethal infection (59). A second study in which an additional treatment was added at day 6 post-challenge protected four of four animals, demonstrating the efficacy of SNALP-encapsulated siRNAs against EBOV.

The use of siRNAs has also been extended to inhibit MARV infection. Fowler et al. used siRNAs against MARV NP, VP30 and VP35 to efficiently reduce levels of these gene transcripts in co-transfection assays in HeLa cells and during MARV infection of Vero cells (60). These data show that MARV infection is also susceptible to RNAi-based targeting; therefore, SNALP-encapsulated siRNAs may achieve similar success treating MARV infection in vivo.

PMOs are another gene targeting method that has shown promise against a number of viruses, including filoviruses (61, 62). PMOs are oligonucleotides modified such that the ribose ring of the nucleotide backbone is replaced with a morpholine ring (63). Also, the anionic phosphodiester linkage is replaced by an uncharged phosphorodiamidate linkage. The presence of an uncharged and not a charged backbone reduces interactions with serum and cellular proteins

***Table I.*** *Comparison of efficacy studies using gene targeting approaches against filoviruses.*

| Therapeutic/gene target | Filovirus | Model | Percentage survival (no. survivors/no. treated) | Ref. |
|---|---|---|---|---|
| SNALP siRNA: L | EBOV | Guinea pig | 80% (8/10)[§] | 58 |
| SNALP siRNA: L, VP35, VP24 | EBOV | Rhesus | 86% (6/7)[#] | 59 |
| siRNA: NP, VP35 & VP30 | MARV | Vero cells | N/A | 60 |
| PMO: VP35* | EBOV | Mouse | 100% (12/12) | 66 |
| PMO: VP35, VP24 & L | EBOV | Mouse, guinea pig & rhesus | 50% (2/4)[£] | 68 |
| PMO plus: VP24, VP35 & NP | EBOV & MARV | Rhesus, cynomolgus[$] | EBOV: 62.5% (5/8)[¶]<br>MARV: 100% (13/13)[¶] | 70<br>70 |

[§]Efficacy results of two studies combined using similar dosing regimens are listed; [#]pooled results from two studies in which the second included and added day of treatment; *represents results of studies using arginine-rich peptide-conjugated PMO; [£]rhesus result is shown; [$]rhesus monkeys were challenged with EBOV and cynomolgus monkeys were challenged with MARV; [¶]efficacy results of post-exposure study not including dose assessment studies.

thought to be responsible for off-target toxicity (64). PMOs are advantageous because the modifications confer high stability and solubility, two favorable qualities for in vivo therapeutic studies (62). Functionally, PMOs differ from siRNAs in that they do not mediate degradation of target messenger RNAs (mRNAs); instead, PMOs typically function by steric hindrance (65). Generally, PMOs are designed to target the 5′ untranslated region, the AUG start codon, splice junctions or critical RNA secondary structural sites; in this way, PMOs reduce protein production levels by impairing mRNA processing or translation.

An initial filovirus study by Enterlein et al. demonstrated that a peptide-conjugated PMO designed against EBOV VP35 offered protection in the EBOV mouse model (66). In a related study, PMOs targeting VP35 either conjugated to an arginine-rich peptide to enhance cellular uptake (67), or left unconjugated, were delivered to mice 24 hours and 4 hours prior to EBOV infection. Of the mice treated with the unconjugated PMO, 75% were protected from lethal infection, while 100% of the mice treated with the conjugated PMO were protected, demonstrating in vivo efficacy for PMOs against EBOV. Next, the efficacy of PMOs targeting VP35, VP24 and L in three different animal models for lethal EBOV infection was investigated (68). Treatment with a PMO targeting L at 24 hours and 4 hours prior to challenge protected only ~30% of treated mice, while PMOs targeting either VP35 or VP24 protected 100% and ~90% of the treated mice, respectively. Administration of all three PMOs in combination provided complete protection when given 24 hours after challenge. In the guinea pig model, protection was seen when the PMOs were administered in combination 4 days post-EBOV infection. These differences in efficacy highlight the inconsistencies that can result when evaluating therapeutics in different animal model species, likely due to a number of factors, including pharmacokinetic variability.

In the third animal model, NHPs were administered either a single PMO against VP35 or a combination of PMOs against VP35, VP24 and L, with treatments from 2 days prior to infection through to 9 days post-EBOV infection. While treatment with the PMO targeting VP35 alone did not protect any of the animals in this study, two of four NHPs given the combination of PMOs were protected from lethal challenge. In this group, a third NHP, although aviremic, succumbed to a secondary bacterial infection on day 15 post-challenge, indicating that the PMO was likely to be successful in inhibiting viral replication.

Recent studies using a more advanced PMO containing a positively charged piperazine linkage within the molecular backbone (PMO*plus*), targeting EBOV VP24, displayed significantly greater efficacy in the EBOV mouse model (69). From these studies, Warren et al. next demonstrated in a double-blind, randomized, crossover trial that PMO*plus* targeting EBOV VP24 and VP35 used in combination (termed AVI-6002) protected five of eight NHPs from lethal EBOV challenge when administered 30-60 minutes after EBOV exposure and continued daily for 10-14 days (70). The use of PMO*plus* was also successfully employed against MARV. PMO*plus* designed against NP and VP24 transcripts of MARV were generated and used in combination (termed AVI-6003) in NHP studies. All animals treated 30-60 minutes after virus exposure and treated daily for 14 days thereafter survived lethal MARV infection. A dose range study showed that when AVI-6003 was administered at 30 mg/kg, all NHPs survived infection, whereas three of five (60%) NHPs in each of the groups treated with 7.5 or 15 mg/kg survived virus challenge. Taken together, these studies offer a promising outlook for the use of PMOs as effective filovirus therapeutics.

## SMALL-MOLECULE INHIBITORS

There have been a number of reports identifying small-molecule inhibitors of filoviruses (Table II). Small-molecule inhibitors are generally defined as low-molecular-weight organic compounds less than 1000 daltons in mass. While the mechanisms of action of some of these inhibitors remain unclear (71-75), others have been described as inhibitors of filovirus entry (76-78). Virus entry offers an attractive target for the discovery of therapeutic inhibitors. Additionally, knowledge of the cellular factors required for entry can contribute significantly to activity-based discovery, as exemplified by studies on entry inhibitors for HIV (79).

In the years since the initial descriptions of filovirus entry events, a number of host factors have been proposed to be important for this process. These factors include: asialoglycoprotein receptor (ASGP-R) (80), integrin beta-1 (81), folate receptor alpha (82), dendritic cell-specific ICAM-3-grabbing non-integrin 1 (DC-SIGN) and liver/lymph node-specific ICAM-3-grabbing non-integrin (L-SIGN) (83), tyrosine-protein kinase receptor TYRO3 (84) and T-cell membrane protein 1 (TIM-1) (85). Although these factors have been demonstrated to have an impact on infectivity, none have been shown to be absolutely required for filovirus entry.

***Table II.*** *List of small-molecule inhibitors of filoviruses.*

| Small molecule | Reported mechanism | Filoviruses targeted | Ref. |
|---|---|---|---|
| FGI-103 | Unknown MARV, RAVV | EBOV, SUDV | 74 |
| FGI-104 | Virion release (TSG-101)[§] | EBOV | 73 |
| FGI-106 | Unknown | EBOV | 71 |
| Chlorin e6 | Unknown | MARV | 72 |
| Compound 7 | Entry | EBOV, MARV | 76 |
| Tetrahydroquinoline | Entry | EBOV[¶] | 77 |
| Oxocarbazate | Entry | EBOV, MARV | 78 |
| LJ-001 | Entry | EBOV, MARV | 114 |
| NSC-62914 | Unknown RAVV | EBOV, MARV, | 86 |
| U-18666A & imipramine | Entry | EBOV, MARV | 87 |
| Compounds 3.0, 3.47 & 3.18 | Entry | EBOV | 93, 95 |
| Carbocyclic 3-deazaadenosine & 3-deazaneplanocin | Replication | EBOV, SUDV, MARV | 75 |
| 1,7-DAAC derivatives 11, 14 & 16 | Unknown | EBOV | |

[§]TSG-101 is the proposed molecular target of FGI-104; [¶]EBOV-GP pseudotyped virions used in these studies.

Recently, two groups identified a novel filovirus entry factor, the Niemann-Pick C1 protein (*NPC1*) (86, 87). Mutation of the *NPC1* gene leads to the metabolic disorder Niemann-Pick disease type C (88). *NPC1* resides on the endosomal and lysosomal membranes and plays a critical role in cholesterol trafficking (88), and has also been shown to be integral in calcium homeostasis and release of HIV-1 particles (89, 90). In both filovirus reports, small molecules were identified that effectively inhibited filovirus entry. Interestingly, one of the inhibitors identified, imipramine, is an FDA-approved antidepressant (86). Although further studies will be required to show that imipramine could be an effective filovirus therapeutic, the implications that one could repurpose existing drugs and shorten development time to licensure are tremendous and worth further investigation.

## TARGETING THE HOST RESPONSE TO INFECTION

Continued investigation of filovirus pathogenesis has elucidated new cellular and viral targets against which effective filovirus therapeutics can be designed. EBOV infection is known to trigger cell surface expression of the procoagulant tissue factor, which binds coagulation factor VII, triggering DIC (54). Geisbert et al. attempted to block this interaction using the recombinant nematode anticoagulant protein C2 (rNAPc2) in studies of EBOV and MARV infection (91, 92). A 33% survival rate was observed in EBOV-infected NHPs treated with 30 µg/kg rNAPc2, along with a marked reduction in coagulopathy and overall decrease in the levels of proinflammatory response mediators. In the MARV study, rNAPc2 appeared to be less effective, as only one of six treated NHPs survived challenge. The authors attributed this reduced survival to the use of the Angola isolate of MARV, where upregulation of tissue factor was less pronounced. Therefore, the mechanism of action for this therapeutic may be less important for the pathogenesis of this MARV isolate.

Other studies which address host response to infection include the investigation of activated protein C activity and inhibition of *S*-adenosylhomocysteine hydrolase (93-95). Protein C is a key biomarker for severe sepsis and was observed to be greatly reduced during EBOV infection of NHPs (92, 96-98). Intravenous infusion of activated protein C to a group of 11 EBOV-infected NHPs administered 30-60 minutes post-infection and continued for 7 days resulted in prolongation of the mean time to death, and survival in 2 of the 11 animals. Another study used mice to investigate *S*-adenosylhomocysteine hydrolase inhibitors with varying success, depending on the day post-infection treatment began (93, 95). These studies emphasize the difficulties of treating filovirus infection during acute stages of the disease.

Filovirus infection has long been shown to result in dysregulation of the host immune response. In particular, filovirus infection triggers the release of massive amounts of cytokines, termed the "cytokine storm". A greater understanding of the mechanism underlying this key pathogenic feature of lethal filovirus infection would aid in the development of effective therapeutics against this process. A recent report examining the cytokine storm observed during lethal influenza virus infection identified the lysophospholipid $S1P_1$ receptor system as playing a key role as a regulator of inflammation. Importantly, pulmonary endothelial cells expressing the $S1P_1$ receptor were shown to lie at the center of a critical regulatory network. The use of $S1P_1$ receptor agonists significantly reduced cytokine release from bronchoalveolar lavage (BAL) and decreased infiltration of macrophages, natural killer cells and neutrophils into the lung (99). Notably, this immunomodulatory function did not affect the generation of an adaptive response and did not seem to alter viral replication. This critical finding should stimulate investigation into the role this system may play during lethal filovirus infection. Because the researchers in this study centered on influenza virus pathogenesis, their study focused on pulmonary endothelial cells. For filovirus-based studies, it will be of interest to analyze more relevant endothelial cell types. Endothelial cells are known to play a prominent role in the pathogenic features of filovirus infection (33, 48, 100). Endothelial cells have been shown to be highly susceptible to filovirus infection in vitro (101-103). However, in vivo, endothelial cells are considered to be more secondary targets of infection (42, 100). The damaging effects of filovirus infection on the endothelium are thought to arise indirectly, due to release of cytokines and other inflammatory mediators from macrophages and other related cell types. It would be of interest to determine if the $S1P_1$ system plays a role during filovirus infection, and moreover, if $S1P_1$ receptor agonists may be used to modulate the "cytokine storm" observed during filovirus infection.

## ANTIOXIDANTS

In addition to the release of proinflammatory cytokines, overproduction and release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is thought to occur during filovirus infection (104). ROS and RNS, such as hydrogen peroxide and nitric oxide, are highly reactive molecules generated as a result of oxygen and nitrogen metabolism (as byproducts or waste products of various necessary reactions), or as part of a cell defense mechanism (105). At low concentrations, these molecules may serve as second messengers in cellular signaling (106). However, excessive production can damage a wide variety of cellular constituents, including proteins, lipids and DNA. This can lead to oxidative stress and pathogenesis in a wide array of diseases, including cancer, neurodegeneration, autoimmune disorders and viral infections (107-110). For a number of conditions though, it is not yet clear if increased oxidative stress is a cause or a consequence of disease (111, 112).

The overproduction of ROS and RNS is likely to be due to impairment or dysregulation of the cellular antioxidant system, which is in place to maintain redox homeostasis. The cellular antioxidant response system is emerging as a key immune regulator during viral infection. Interestingly, in a murine model of septic shock it was demonstrated that the cellular antioxidant system plays a key role in modulating sepsis (113). Relatively little is known about the impact of cellular redox imbalance on the underlying pathology of filoviruses. A recent report identified an antioxidant small molecule, NSC-62914, as being capable of effectively inhibiting filovirus replication. Importantly, NSC-62914 showed efficacy in studies using a mouse model of both EBOV and MARV (114). It should be noted that a definitive link between the antioxidant function and antiviral function of the small molecule could not be concluded in this study. Nevertheless, the extensive use of antioxidants as a therapeutic option for a number of viral infections, including influenza and hepatitis C virus (HCV) (115, 116), coupled with the emerging role of the antioxidant response system in immune regulation, indicates that

this area warrants further exploration towards treating filovirus infections.

## CONCLUSION

A significant step towards licensure of a filovirus therapeutic is to show protection in the NHP model of filovirus infection. This is largely because filovirus disease in NHPs is thought to resemble human infections. In this regard, the major advancements in filovirus therapeutics to date have come from the use of vaccine platforms, which includes the use of live virus, replicon-based vectors, as well as protein-based vaccines (117, 118). Comparatively, the use of small-molecule inhibitors and gene targeting approaches has lagged behind, although RNAi and antisense-based approaches have recently emerged as effective filovirus therapeutics in NHPs. Studies using either SNALP-encapsulated siRNA or PMOs demonstrate that these gene targeting strategies hold a great deal of promise alongside the aforementioned vaccine platforms. Similarly, in the near future we expect significant strides to be made with small-molecule inhibitors, in particular, for those small molecules repurposed for use against filoviruses. These small molecules will have a shorter time to begin testing in NHPs, since critical information regarding the absorption, distribution, metabolism, excretion and toxicity (ADMET) properties is already known (86).

An emerging tool in filovirus research is the increased use of chemoinformatics coupled with advanced high-throughput screening. These technologies will greatly increase the potential of identifying novel small-molecule inhibitors for in vivo studies. We also anticipate that future progress in the development of small-molecule inhibitors will be enhanced by the continued understanding of the molecular mechanism underlying pathogenesis. This includes identification of novel pathways such as the $S1P_1$ system and antioxidant response system, which may play key regulatory roles in filovirus disease progression. Should a link be established between these systems and filovirus disease progression, a trove of potential small-molecule inhibitors will be available for use against filoviruses, as there are a host of well-characterized small molecules known to modulate these systems. Taken together, the significant achievements gained in gene targeting studies along with the continued identification of novel and effective inhibitors will result in promising lead candidates to treat filovirus infections.

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## DISCLOSURES

The authors state no conflicts of interest.

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