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Current status of small molecule drug development for Ebola virus and other filoviruses

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Abstract

The filovirus family includes some of the deadliest viruses known, including Ebola virus and Marburg virus. These viruses cause periodic outbreaks of severe disease that can be spread from person to person, making the filoviruses important public health threats. There remains a need for approved drugs that target all or most members of this virus family. Small molecule inhibitors that target conserved functions hold promise as pan-filovirus therapeutics. To date, compounds that effectively target virus entry, genome replication, gene expression and virus egress have been described. The most advanced inhibitors are nucleoside analogs that target viral RNA synthesis reactions.

The filovirus family.

Filoviruses, zoonotic pathogens associated with severe disease in humans, are filamentous, enveloped viruses with non-segmented, negative-sense RNA genomes [1]. Included in filovirus family is the genus *Ebolavirus*, which has six species. Among these, Zaire ebolavirus (EBOV), Sudan virus (SUDV) and Bundibugyo virus (BDBV) have caused substantial outbreak with significant morbidity and mortality in humans. *Marburgvirus* is another genus with members that have caused that includes Marburg virus (MARV) and Ravn virus (RAVV). *Cuevavirus*, which contains Lloviu virus (LLOV) and proposed genus *Dianlovirus*, which contains a single member, Měnglà virus (MLAV), have not to date been associated with human disease [2–4]. LLOV and MLAV, both identified in bats, have not been isolated or cultured and their significance with regard to human health is unknown.

The largest filovirus outbreak on record was caused by EBOV in West Africa between 2013–2016. This resulted in more than 28,000 infections, more than 11,000 deaths and the export of infected cases to the United States and Europe [5]. In pregnant women the fatality rate is estimated to be 70%, and survivors are known to exhibit persistent infection with virus residing in immune privileged sites, including the eye and testes [6–10]. The only treatments available were supportive care and experimental therapies, hampering patient treatment and

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leaving healthcare workers at severe risk. The West Africa epidemic reinforced the threat posed by the filovirus family and demonstrated that in addition to being a bio-terrorism threat, emergences from natural sources can have a profound public health impact.

Because of their extreme virulence, fully-replication competent filoviruses are studied in Biosafety Level 4 (BSL4) containment. This limits the number of investigators who have direct access to virus and makes screening of antiviral against live virus challenging, even for those investigators with BSL4 access. Therefore, much effort has been devoted to targeting specific steps in the virus replication cycle, such as viral entry, viral RNA synthesis and virus assembly and release, that can be reconstituted in transfection-based studies that do not require live virus. This approach can facilitate the discovery of small molecules that target specific viral functions that must then be tested for efficacy against live virus in cell culture and animal models.

Summary of relevant filovirus biology.

The filovirus genome is approximately 19 kilobases in length and encodes up to nine translation products from seven separate transcriptional units [1,11]. These genes encode the viral nucleoprotein (NP), viral protein of 35 kDa (VP35), VP40, a type I transmembrane glycoprotein (GP), VP30, VP24, and the large protein (L), which is the viral polymerase. Members of the *Ebolavirus* genus, and also likely LLOV, produce secreted forms of the GP protein [1,12,13] (Fig 1A).

Viral entry is mediated by GP which acts as an attachment factor and mediates fusion of viral and host cell membranes within an endosomal compartment [14] (Fig 1B). The viral genome is released into the cytoplasm as a ribonucleoprotein complex. This serves as the template for the RNA synthesis reactions that replicate the viral genomic RNA and transcribe the mRNAs that lead to viral gene expression. Replication requires NP, which associates with the viral genomic and antigenomic RNAs throughout the course of infection; VP35, a non-enzymatic cofactor for the viral RNA-dependent RNA polymerase that also serves as a potent suppressor of innate antiviral signaling pathways and L, which possesses all the enzymatic activities required for viral transcription and genome replication, including RNA-dependent RNA polymerase (RdRp) activity, guanyltransferase and methyltransferase activities [15,16]. Viral transcription (mRNA synthesis) involves the production of distinct 5’-capped, 3’polyadenylated mRNAs from each of the viral genes and requires, in addition to NP, VP35 and L, the VP30 protein [16] (Fig 1B). Co-transfection of these four viral proteins with a model viral genomic RNA can recapitulate the filovirus RNA synthesis machinery in cell-based “minigenome” assays in biosafety level 2 (BSL2), enabling the study of filovirus RNA synthesis [17–20]. In addition to the required viral proteins, host factors modulate viral RNA synthesis through interaction with viral factors, however, a complete understanding as to how host factors contribute to viral RNA synthesis remains elusive [21–23].

Other viral functions include filovirus assembly and release [24]. The VP40 matrix protein drives the membrane budding events that lead to release of new virus particles. GP is incorporated into the membrane of viral particles and enhances budding. Viral ribonucleoproteins (RNPs) that contain genomic RNA, NP, VP35, VP30 and VP24 are

recruited into the budding particles. In addition to playing roles in replication and assembly, several filovirus proteins counteract host innate antiviral defenses [25]. The filovirus VP35 proteins block interferon (IFN)-α/β production and the VP24 proteins of *Ebolavirus* and *Cuevavirus* genus members and the VP40 proteins of the *Marburgvirus* genus block IFN-induced antiviral signaling [15,26–34]. *Marburgvirus* VP24 proteins also modulate host antioxidant response pathways through interaction with the host protein Keap1 [35–37]. While these viral functions can be studied independently by transfection based assays, inclusion of the viral genes for VP40, GP and VP24 into the model viral genomic RNA results in an advanced system that produces replication and transcription-competent virus-like particles (trVLPs), allowing for BSL2 study of most viral lifecycle steps, including entry and budding, in addition to RNA synthesis [38].

Status of Promising Anti-Filovirus Approaches.

The typical progression of anti-filovirus therapeutics is demonstration of efficacy in cell culture, then in mice, followed by guinea pigs and then non-human primates (NHPs), the "gold standard" for efficacy in animals [39]. Historically, NHPs have proven much more difficult to protect than rodents. The focus of this review is small molecule inhibitors of EBOV and other filoviruses, which are summarized in Figure 1 and Table 1. It is nonetheless important to recognize that monoclonal antibodies and nucleic acid-based therapeutics have also successfully protected NHPs from lethal filovirus challenge. A notable achievement was the finding that the three monoclonal antibody cocktail ZMAPP could protect NHPs from lethal challenge even after the onset of clinical symptoms [40]. Antibody-based approaches are sufficiently promising that three such treatments are being investigated in a clinical trial in the 2018–2019 EBOV outbreak in the Democratic Republic of Congo. These are ZMAPP (Mapp Biopharmaceutical, Inc.), Mab 114 (National Institute of Allergy and Infectious Diseases) and the three monoclonal antibody cocktail REGN-EB3 (Regeneron).

Targeting filovirus RNA synthesis.

Targeting viral RNA synthesis reactions shows substantial promise, including the targeting of viral RNA polymerase function by nucleoside analogues and small molecules that affect viral protein expression, stability and post-translational modifications necessary for viral replication.

Nucleoside analogues BXC4430, an adenosine analogue, GS-5734 (Remdesivir, Gilead, USA), a monophosphoramidate prodrug of an adenosine analogue and favipiravir (T-705, Toyama Chemical, Japan), a synthetic guanidine nucleoside analogue, exhibit anti-filovirus activity, likely through inhibition of viral polymerase activity [41–43]. BXC4430 was the first small molecule demonstrated to protect non-human primates from lethal filovirus challenge, even when administered up to two days post-infection [43]. This compound has progressed to Phase I clinical trials (URL:ClinicalTrials.gov Identifier:NCT02319772 and NCT03800173).

GS-5734 exhibits antiviral activity against a number of RNA viruses and has been shown to inhibit respiratory syncytial virus (RSV) and hepatitis C virus (HCV) polymerases, with greater selectivity for the viral polymerase than the cellular [42,44,45]. GS-5734

demonstrated protection of rhesus macaques from EBOV challenge across several dosing regimens, including complete protection from lethal disease of animals that received GS-5734 beginning three days post infection [42]. Interestingly, in macaques, administration led to distribution in tissues such as testes, epididymis, eyes and brain where EBOV may reside after recovery from illness. GS-5734 was provided to some human patients infected during the West Africa epidemic, and has been used in the 2018–2019 outbreak in DRC under an emergency use protocol for experimental medical interventions [7,46] (WHO; URL:https://www.who.int/ebola/drc-2018/treatments-approved-for-compassionate-use/en/). It is also currently in clinical trials, including a study to evaluate treatment of male survivors of Ebola virus disease (EVD) with persistent EBOV in their semen (URL:ClinicalTrials.gov Identifier:NCT02818582).

Favipiravir has broad spectrum activity against a number of RNA viruses and has been studied in Phase 3 clinical trials in Japan and the United States, with approval in Japan for treatment of influenza virus infection (URL:ClinicalTrials.gov Identifier:NCT02008344; Toyama Chemical Co Ltd.; URL:https://www.toyama-chemical.co.jp/eng/news/news140324e.html). It likely acts as a “pseudo purine”, inhibiting influenza virus RdRp activity with selectivity towards viral over cellular polymerases [47]. Favipiravir demonstrated protection of type I interferon receptor (IFNAR) knockout mice and immunocompetent C57BL/6 mice from challenge with EBOV and mouse-adapted EBOV (MA-EBOV), respectively [41,48,49]. When tested in macaques, favipiravir administered orally once or twice daily resulted in only one survivor out of eighteen EBOV infected animals, although delayed time to death and reduced viral levels were documented [41]. In contrast, intravenous administration of favipiravir led to five of six MARV infected animals surviving, with reduced viral loads and symptoms of MARV disease in survivors and delayed time to death for the animal that succumbed. Two clinical trials examined favipiravir as a treatment for EVD during the West Africa epidemic [50,51]. Due to the design of the trials, definitive conclusions could not be made. However, favipiravir treatment correlated with decreased viremia in subjects with low initial viral load, improved survival rates and reduced symptoms. Together, these studies suggest that continued examination of favipiravir as an anti-EBOV therapy is warranted.

Nucleoside analogues also exhibit antiviral activity by mechanisms other than direct inhibition of viral polymerase activity. Carbocyclic nucleosides, such as 3-deazaneplanocin A, inhibit replication of a number of negative sense RNA viruses including filoviruses [52–57]. The mechanism of action is thought to be inhibition of cellular S-adenosylhomocysteine (SAH) hydrolase (SAHase), which breaks down SAH produced from S-adenosylmethionine (SAM), a molecule required for macromolecular methylation reactions. Inhibition of SAHase raises intracellular SAH levels, blocking cellular methylation reactions via a feedback inhibition mechanism. This causes diminished methylation of viral mRNAs 5’ cap moieties and impairs viral protein synthesis [58]. 3-deazaneplanocin A has been demonstrated to exhibit anti-EBOV activity *in vivo*, as it can protect mice from EBOV challenge [52].

Several other nucleoside analogues with anti-EBOV activity have been identified through antiviral screens using EBOV or minigenome assays confirmed in cell culture with EBOV.

These include the cytidine analogues β-d-N4-hydroxycytidine (NHC) and azacytidine and the uridine analogue 6-azauridine [17,18,59,60]. Further study is required to demonstrate mechanism of action and efficacy in animal models for these compounds.

A number of non-nucleoside compounds have also been demonstrated to inhibit filovirus replication in cell culture. Benzoquinoline compounds were identified by minigenome assay as inhibitors of EBOV RNA synthesis, with activity shown against a variety of RNA virus families [61]. While their mechanism of action is unknown, the broad-spectrum activity suggests a host target. Hsp90 inhibitors demonstrate inhibition of EBOV in cell culture, likely through the destabilization of EBOV L [23,62]. It has also been demonstrated that inhibition of polyamine biosynthesis, such as by 2-difluoromethylornithine (DFMO), and hypusination, by *N*1-guanyl-1,7-diamineheptane (GC7) and ciclopirox, reduces EBOV replication [63,64].

Inhibitors of the host enzyme dihydroorotate dehydrogenase (DHODH), which has a role in *de novo* pyrimidine biosynthesis, have broad-spectrum antiviral activity that includes inhibition of EBOV in cell culture [65,66]. The anti-EBOV activity of DHODH inhibitors such as GSK983 and brequinar is related to depletion of pyrimidine pools [65]. Interestingly, a genetic screen also identified de novo pyrimidine biosynthesis as critical for EBOV replication [67]. Although DHODH inhibitors have been used clinically for other applications, *in vivo* studies have not demonstrated convincing antiviral activity, possibly because uracil is available systemically *in vivo* to feed the salvage pathway, overcoming the block to the de novo pyrimidine synthesis pathway [68–70].

Filovirus replication can also be targeted through the VP30 protein, which is required for EBOV and MARV growth and plays roles in viral mRNA synthesis. This function is regulated by VP30 phosphorylation, with dephosphorylated VP30 promoting viral mRNA synthesis and phosphorylated VP30 promoting viral genome RNA replication. Compounds that prevent VP30 dephosphorylation, such as Okadaic acid (OA) and 1E7–03, impair virus growth in cell culture [71–73].

Another strategy being pursued is the targeting of protein-protein interactions involved in filovirus RNA synthesis. This has been enabled by the increasing numbers of virus-virus and virus-host protein-protein interactions that have been identified and characterized by structural, biophysical and molecular biology methods [1,74,75]. In one example, a fluorescence polarization assay was developed around the interaction of NP and the NP binding peptide (NPBP) derived from VP35, an interaction critical for viral RNA synthesis [75]. Screening for inhibitors of the interaction identified Tolcapone, an FDA-approved drug that is used in the treatment of Parkinson’s disease [76,77]. Tolcapone was demonstrated to impair EBOV replication in cell culture. As the NP:NPBP interaction site is well-conserved among filoviruses, the NP:NPBP interaction has potential as a pan-filovirus target [77].

Entry Inhibitors.

There has been substantial effort devoted to developing small molecule inhibitors of EBOV entry. The entry process itself has been studied in depth and is relatively unique [78]. A number of cell surface molecules, including lectins and phosphatidyl serine (PS)-binding

proteins have been described to mediate virus attachment to the cell surface, via interactions with GP or PS on the virus surface [78]. Uptake is by macropinocytosis or a macropinocytosis-like process [79–82]. Within endosomes, GP undergoes cleavage by proteases cathepsin B and cathepsin L, although EBOV replication in both cell culture and mouse models may not absolutely require that both these proteases be active [83–89]. Cleaved GP then interacts with host protein Niemann–Pick C1 (NPC1) within the endosomal lumen [90–92]. This interaction is necessary for fusion of viral and endosomal membrane and release of virus particles. Each of these steps, as well as cellular functions associated with these steps, are potential targets for therapeutic intervention.

Compounds directly targeting GP or the viral membrane.

A benzodiazepine derivative called compound 7 was identified in a screen for entry inhibitors through the use of GP pseudotyped lentiviruses [93]. Compound 7 demonstrated selectivity towards inhibition of EBOV and MARV in cell culture over other RNA and DNA viruses and was shown to directly bind GP [93]. In contrast, LJ001, a rhodanine derivative identified in a screen for inhibitors of Nipah virus entry, was demonstrated to have broad-spectrum activity against enveloped but not non-enveloped viruses. It was shown to bind to lipid membranes, acting as a type II photosensitizer modifying unsaturated phospholipids, negatively affecting enveloped virus entry without significant host cell cytotoxicity[94]. LJ001 was also shown to inhibit EBOV replication in cell culture and to protect 80 percent of mice challenged with MA-EBOV [95].

Arbidol is a small molecule used clinically in Russia and China to prevent and treat influenza virus infections [96]. It has broad-spectrum antiviral activity in cell culture, including anti-EBOV activity that is suggested to be through inhibition of EBOV entry [97]. This may be through the capacity of arbidol to bind lipid membranes, but as it has also been demonstrated to directly bind influenza A virus hemagglutinin protein preventing fusion, the possible interaction with filovirus GPs may warrant exploration [98,99].

Inhibitors of macropinocytosis.

Compounds that inhibit macropinocytosis, such as ethylisopropylamiloride (EIPA), an inhibitor of the Na^+/H^+ exchanger that specifically inhibits macropinocytosis, PKC inhibitor rottlerin, actin polymerization inhibitor latrunculin A, and PI3-kinase inhibitor wortmannin all inhibit EBOV entry [79,82]. Recent screening with MARV GP pseudotyped VSV and retrovirus particles identified 17 compounds able to inhibit MARV and EBOV in cell culture, two of which were novel macropinocytosis inhibitors [100].

Inhibitors of cathepsins.

The proteolysis of EBOV GP by cathepsins B and L, and the critical role of this activity in EBOV entry, was demonstrated in part through the use of pharmacological inhibitors of proteases. These inhibitors include the cysteine-serine protease inhibitor leupeptin, cysteine protease inhibitors E64, E64a, E64d, K11777 and K11777-derivatives, cathepsin B inhibitors CA074 and CA074Me and cathepsin L inhibitor III [83,84,101–105]. Several more studies have been undertaken to identify inhibitors of cathepsin B and L cleavage of GP and inhibition of filovirus entry [106–108]. These include the natural product aloperine

and its derivatives which target cathepsin B and the glycopeptide antibiotic teicoplanin that is suggested to inhibit cathepsin L. Finally, it should be noted that cathepsins B and L are activated by low pH and inhibitors of endosomal acidification also inhibit filovirus entry [109,110].

Inhibitors that target NPC1 interaction with GP or mimic the phenotype of NPC1 deficiency.

NPC1 was initially identified as an essential receptor for EBOV entry by both a genetic and a chemical screen [90,91]. NPC1 is an endosomal and lysosomal cholesterol transporter and mutations in NPC1 are associated with Niemann–Pick disease, a neurovisceral atypical lysosomal lipid storage disorder where cholesterol and sphingolipids accumulate in lysosomes [111]. A benzylpiperazine adamantane diamide compound, called 3.0, and an analogue, 3.47, identified in the chemical screen caused cholesterol accumulation in cells and were demonstrated to interact with NPC1, inhibiting binding of cleaved GP [91]. Although the cholesterol transport function of NPC1 is not required for EBOV entry, compounds that mimic NPC1 deficiency in cells, including imipramine and U18666A, the latter of which also binds NPC1, have been shown to block EBOV entry [90,112,113]. However, while imipramine and U18666A treatment of mice infected with MA-EBOV led to lower viral replication, no significant protection was demonstrated [114].

Compounds inhibiting late steps in the entry process.

Selective estrogen receptor modulators (SERMs), including clomiphene and toremifene, were identified during screening of FDA-approved drugs as inhibitors of EBOV entry in cell culture [115–117]. When tested in mice, clomiphene protected 90% of animals and toremifene protected 50% of animals from death [115]. Mechanistically, the compounds function in cells lacking estrogen receptor, suggesting an off-target effect and were shown in one study to inhibit a late step in EBOV entry, such that fusion does not occur, although in another, toremifene was found to bind and destabilize GP [115,118].

Repurposing of FDA drugs is complicated by an inability to achieve *in vivo* levels sufficient for anti-EBOV activity, therefore combinations of inhibitors have been assessed for efficacy [119]. Two 3-drug combinations, toremifene-mefloquine-posaconazole and toremifene-clarithromycinposaconazole, were identified as being active at clinically achievable concentrations. Mechanistic studies suggested all inhibit NAADP-AM stimulated lysosomal calcium release, while posaconazole inhibits NPC1 function and posaconazole, toremifene and mefloquine inhibit acid sphingomyelinase activity.

EBOV entry requires endosomal calcium channels known as two-pore channels (TPCs) [120]. Inhibition of TPCs by genetic or pharmacological approaches, including the FDA-approved drugs verapamil, nimodipine and diltiazem, as well as the natural product tetrandrine, prevent EBOV escape from endosomes, thereby aborting infection [110,120–122]. Tetrandrine, the most potent of these, was demonstrated to protect mice from lethal challenge with mouse-adapted EBOV [120].

Other genes potentially important for EBOV entry were described in the genetic screen that identified NPC1, including phosphatidylinositol-3-phosphate 5-kinase (PIKfyve) [90]. Based on this, the small molecule apilimod, which inhibits PIKfyve, was examined and

demonstrated potent inhibition of EBOV and MARV entry in human macrophages [123]. The mechanism of inhibition appears to be impaired trafficking of incoming virus to sites where NPC1 resides and membrane fusion takes place [123]. Apilimod has been well-tolerated in phase I and II clinical trials as an interleukin-12/23 inhibitor for the treatment of Crohn's disease, psoriasis, and rheumatoid arthritis [124–127]. Given these activities, it will be of interest to determine whether the antiviral activity of apilimod outweighs its immune suppressing activities in the context of filovirus infection *in vivo*.

Other entry inhibitors.

A combination of the kinase inhibitors genistein and tyrphostin AG1478 was demonstrated to inhibit infection by EBOV and MARV GP-pseudotyped VSV and to inhibit EBOV growth, although the mechanism remains to be elucidated [128].

Inhibitors of EBOV egress.

The VP40 protein of filoviruses serves as the major matrix protein that is responsible for the budding of new virus particles from the cell surface. Budding is facilitated by the interaction of proline-rich "late domain" motifs on VP40 with components of the host cell vacuolar protein sorting (vps) pathway, such as Tsg101 and Nedd4 [129–131]. An *in silico* screen using the NMR structure of the PTAP peptide-binding pocket in human tsg101 identified a compound, 5539–0062, that was demonstrated to inhibit the interaction between tsg101 and EBOV VP40 and to prevent budding of VP40 from cells in transfection studies [132]. High concentrations of compound were needed; however, the inhibitory concentrations were not cytotoxic [132]. A second *in silico* screen using the structure of a WW domain of Nedd4 in complex with a PPxY motif identified compound 1 that inhibits MARV VP40 budding [133]. Structure/activity studies led to the generation of compounds with enhanced potency and inhibition of both MARV and EBOV VP40 interaction with Nedd4 and budding [133,134]. This inhibition extended to other viruses where matrix proteins rely on late domain-Nedd4 interactions for budding, including VSV and rabies virus [133].

Conclusions.

The development of therapeutic antibodies and small molecule inhibitors that protect non-human primates from EBOV challenge is a major milestone as is advancement of therapeutics into clinical trials. Given that none is yet approved for human use and the facts that propensities of these approached to elicit resistance, continued efforts at drug development for filoviruses remains a necessity. Small molecule approaches targeting conserved viral functions would seem to offer the greatest possibility for pan-filovirus efficacy. Therefore, small molecule drug development should be a priority.

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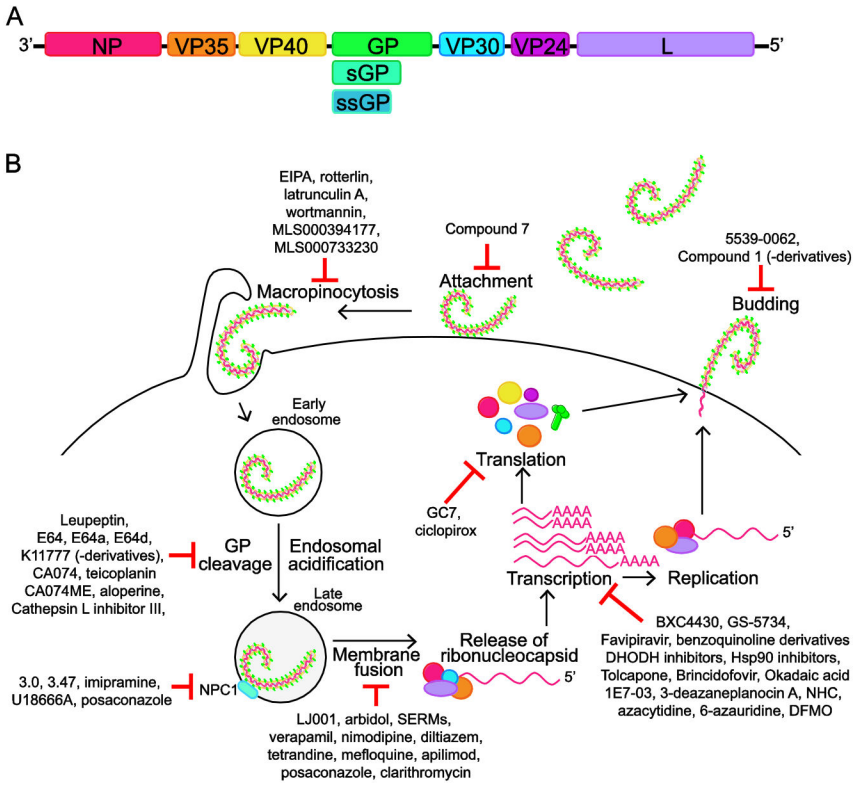

Figure 1. Filovirus genome, replication cycle and small molecules inhibitors.

A. Schematic of filovirus genome. The negative-sense RNA genome has seven transcriptional units that encode for the nucleoprotein, NP; viral protein 35, VP35; VP40; glycoprotein/soluble glycoprotein, GP/sGP (sGP is not encoded by Marburg virus); VP30; VP24; Large protein, L (viral polymerase). Note that sGP and ssGP is produced by members of the *Ebolavirus* genus, and predicted to be produced by LLOV. Genome schematic is not to scale. **B.** Schematic of the steps in the filovirus lifecycle. GP mediates attachment of the filovirus to the surface of the cell. The virus is then taken up by macropinocytosis. Following acidification of the endosome, cathepsins B and L cleave GP, a requirement for its interaction with the host protein NPC1 that facilitates fusion of viral and endosomal membranes. Endosomal calcium channels, known as two-pore channels (TPCs), play a role in trafficking the virus particle to the site of membrane fusion. Following fusion, the ribonucleocapsid is released into the cytoplasm where 5'-capped, 3'polyadenylated mRNAs are transcribed for each viral gene and a copy of the full-length genomic RNA is produced, which acts as a template for synthesis of new negative-sense viral genomes. Transcription requires NP, VP35, VP30 and L, while replication does not need VP30. Viral proteins are translated from the viral mRNAs and new viral particles are formed at the cell surface. VP40 drives viral budding and is assisted by GP. Viral ribonucleoproteins containing genomic RNA, NP, VP35, VP30 and VP24 are incorporated into the budding particles. The steps in the filovirus lifecycle are potential targets for therapeutic intervention; small molecules that target these processes are noted on the schematic. Greater detail on the filovirus lifecycle and these small molecules can be found in the review.

Table 1.

Status of small molecule drugs developed for filovirus infection.

Targeted step of virus lifecycle	Small Molecule	MOA[a]	Efficacy *in vitro*	Efficacy *in vivo*	Clinical Trial[b]
RNA synthesis	BCX4430[43]	Inhibition of viral RdRp - chain terminator	IC_{50} 3.4–11.8 μM[c,d,e,f] IC_{90} 10.3–25.4 μM[c,d,e,f]	Protection in mice, guinea pigs. NHPs: 15 mg/kg, twice daily via i.m.[g] confers protection against MARV when administered up to 48hr post-infection.	Phase I (NCT02319772, completed; NCT03800173, recruiting)
	GS-5734[42]	Inhibition of viral RdRp - chain terminator	IC_{50} 0.06–0.14 μM[d,h,i] IC_{90} 0.18–0.41 μM[d,h,i]	Confers complete protection in NHPs against EBOV when administered 72hr post-infection at a 10 mg/kg loading dose followed by 3 or 10 mg/kg daily dose i.v.[j]	Phase II (NCT02818582, recruiting)
	Favipiravir[41,48,49]	Either inhibition of viral RdRp and/or causes lethal mutagenesis	IC_{50} 67 μM[d,k] IC_{90} 110 μM[d,k]	Complete protection of IFNAR knockout mice, immunocompetent C57BL/6 mice. NHPs: Three p.o.[l] dosing regimens (400 mg/kg loading dose at day −3 followed by 200 mg/kg daily, 250 mg/kg loading dose at day 0 followed by 150 mg/kg twice daily, 125 mg/kg loading dose at day 0 followed by 75 mg/kg twice daily) protected 1 of 18 EBOV infected NHPs, despite plasma levels above EC_{50} values. I.V. dosing of a 250 mg/kg loading dose followed by 150 mg/kg twice daily protected 5 of 6 MARV infected NHPs.	Phase III (influenza, NCT02008344, completed) Phase II (EBOV, NCT02329054, completed; NCT02662855, completed)
	3-deazaneplanocin A[52,53]	Inhibition of SAH hydrolase	IC_{50} 2 μM[d,k]	Complete protection of BALB/c mice against MA-EBOV[aa] when treated once with 1 mg/kg up to 48hr post-infection.	N/A
	(β-d-N4-hydroxycytidine (NHC)[60]	Unknown	IC_{50} 3 μM[d,k]	N/A	N/A
	Azacytidine[17]	Unknown	IC_{50} 4 μM[m,u] 86% inhibition at 50 μM[d,k]	N/A	N/A

Targeted step of virus lifecycle	Small Molecule	MOA[a]	Efficacy *in vitro*	Efficacy *in vivo*	Clinical Trial[b]
	6-azauridine[18]	Unknown	IC_{50} 5 μM[n,p] and 14 μM[m,p] 98% inhibition at 80 μM[c,k] 99% inhibition at 80 μM[d,k]	N/A	N/A
	Benzoquinoline compounds[61]	Unknown	IC_{50} 0.5–5.6 μM[m,u] >2 log reduction of viral titer at 1 μM[d,k]	N/A	N/A
	HSP90 inhibitors (such as geldanamycin, 17AAG and radicicol) [23]	Destabilization of EBOVL	Geldanamycin: IC_{50} 1.6 μM[d,k] 17AAG: IC_{50} 5.3 μM[d,k] Radicicol: IC_{50} 1.7 μM[d,k]	N/A	N/A
	Inhibitors of polyamine biosynthesis (such as DFMO)[63,64]	Defect in EBOV RdRp dependent mRNA accumulation	DFMO: 90% inhibition at 500 μM[m,p]	N/A	N/A
	Inhibitors of hypusination (such as GC7 and ciclopirox)[63,64]	Ineffective translation of EBOV mRNA	GC7: 85% inhibition at 10 μM[m,p] Ciclopirox: >2 log reduction of viral titer at 30 μM[c,d,o]	N/A	N/A
	DHODH inhibitors (such as GSK983 and Brequinar)[65]	Inhibition of *de novo* pyrimidine synthesis	GSK983: IC_{50} 0.007 μM[m,u] and <0.02 μM[d,f] Brequinar: IC_{50} 0.15 μM[m,u] and 0.1 μM[d,f]	N/A	N/A
	Inhibitors of VP30 dephosphorylation (such as Okadaic acid (OA) and 1E7–03)[71,72]	Inhibition of viral transcription as phosphorylated VP30 does not participate in transcription	OA: IC_{50} 0.13μM OA[m,p] >90% inhibition at 0.08 μM[d,p,*] 1E7–03: 200-fold reduction of viral titerat 10 μM[d,k]	N/A	N/A
	Tolcapone[77]	Inhibition of NP:NPBP interaction, necessary for polymerase activity	IC_{50} 2 μM[q] At 10 μM, >100 fold decrease at MOI 0.01 and >5 fold reduction at MOI 2[d,k]	N/A	N/A
Virus Entry	Compound 7[93]	Direct interaction with GP	IC_{50} 10 μM[d,k]and 12 μM[c,k]	N/A	N/A
	LJ001 [94,95]	Inhibition of virus fusion - type II photosensitizer that modifies unsatu rated phospholipids, negatively impacting viral membrane	0.5 μM < IC_{50} < 1 μM[c,d,k]	At 20 μM, protects 80% of mice infected with MA-EBOV.	N/A

Curr Opin Virol. Author manuscript; available in PMC 2020 April 16.

Targeted step of virus lifecycle	Small Molecule	MOA[a]	Efficacy *in vitro*	Efficacy *in vivo*	Clinical Trial[b]
	Arbidol[97]	Unknown	IC_{50} 2.7 μM[d,o]	N/A	N/A
	EIPA[79]	Inhibition of macropinocytosis prevents cellular uptake of virus	~50–75% inhibition at 100 μM[r,f,k,*] ~70% inhibition at 200 μM[d,k,*]	N/A	N/A
	Latrunculin A[79]	Inhibition of macropinocytosis prevents cellular uptake of virus	~30–65% inhibition at 0.5 μM[r,f,k,*] ~40% inhibition at 0.5 μM[d,k,*]	N/A	N/A
	Wortmannin[79]	Inhibition of macropinocytosis prevents cellular uptake of virus	~50% inhibition at 0.1 μM[r,f,k,*]	N/A	N/A
	Rottlerin[82]	Inhibition of macropinocytosis prevents cellular uptake of virus	>50% inhibition at 2.5 μM[r,k]	N/A	N/A
	Leupeptin[105]	Inhibition of cysteine-serine protease, inhibits GP proteolysis	>95% inhibition at 10 μM[k,s,*]	N/A	N/A
	E64, E64a, E64d[84]	Inhibition of cysteine protease, inhibits GP proteolysis	E64d: >90% inhibition at 50 |jM[k,s,*]	N/A	N/A
	K11777 and derivates[101]	Inhibition of cysteine protease, inhibits GP proteolysis	K11777: 0.87–5.91 nM[u,s,t,w] Derivatives: 0.1–2.69 nM[u,s]	N/A	N/A
	CA074[83], CA074Me[84]	Cathepsin B inhibitor	CA074: ~90% inhibition at 10 μM[s,k,*] >10-fold decrease at 80 μM[d,k,*] CA074Me: >80% inhibition at 0.5 μM[s,k,*]	N/A	N/A
	Cathepsin L inhibitor lll[108]	Cathepsin L inhibitor	IC_{50} 7 μM[s,u]	N/A	N/A
	Aloperine and derivatives[107]	Cathepsin B inhibitor	Derivative 2e: IC_{50} 4.8 μM[u,s] and 7.1 μM[u,t]	Aloperine derivative 2e administered half by i.v. (50 μg) and half by i.p.[v] (50 μg) on day of challenge with pHIV-EBOVGP-Fluc or pHIV-MARVGP-Fluc reduced bioluminescence by 58% 4 days post-infection and 45% 5 days post-infection, respectively.	N/A
	3.0 and 3.47[91,114]	Interacts with NPC1, inhibiting binding of cleaved GP	3.0: 1 μM < IC_{50} < 10 μM[k,s] >99% inhibition at 20 μM[d,k] 3.47: 0.01 μM < IC_{50} < 0.1 μM[k,s]	3.47: Mice treated i.p. with 1, 5 or 25 mg/kg daily, showed no protection from MA-EBOV infection.	N/A

Targeted step of virus lifecycle	Small Molecule	MOA[a]	Efficacy *in vitro*	Efficacy *in vivo*	Clinical Trial[b]
	lmipramine[114]	Unknown, mimics NPC1 deficiency	~50% inhibition at 10 μM[c,d,x]	Mice treated i.p. with 20 mg/kg daily or every other day showed no significant protection from MA-EBOV infection, although lower viral replication at day 3 and 5 was detected.	N/A
	U18666A[90,114]	Unknown, mimics NPC1 deficiency	~99% inhibition at 10 μM[s,k]	Mice treated i.p with 2 mg/kg daily or every other day showed no significant protection from MA-EBOV infection, although lower viral replication at day 3 was detected.	N/A
	Toremifene[115]	Unknown, off-target effect	IC_{50} 0.973–6.17 μM[c,d,e,k]	Mice treated i.p. with 60 mg/kg on day 0 and 1 and every other day after showed 50% protection from MA-EBOV infection.	N/A
	Clomiphene[115]	Unknown, off-target effect	IC_{50} 3.83–11.1 μM[c,d,e,k]	Mice treated i.p. with 60 mg/kg on day 0 and 1 and every other day after showed 90% protection from MA-EBOV infection.	N/A
	Mefloquine[116]	Unknown	IC_{50} 2.73 μM[f,r]	N/A	N/A
	Posaconazole[116]	Unknown	IC_{50} 7.69 μM[f,r]	N/A	N/A
	Clarithromycin[116]	Unknown	IC_{50} 4.53 μM[f,r]	N/A	N/A
	Verapamil[121]	Prevents EBOV escape from endosome	>80% inhibition at 60 μg/ml[s,t,y]	N/A	N/A
	Nimodipine[120]	Prevents EBOV escape from endosome	IC_{50} ~25 μM[d,f,*]	N/A	N/A
	Diltiazem[120]	Prevents EBOV escape from endosome	IC_{50} ~25 μM[d,f,*]	N/A	N/A
	Tetrandrine[120]	Prevents EBOV escape from endosome	IC_{50} 55 μM[d,f]	Mice treated i.p. with 30 mg/kg on day 0 and every second day showed 75% protection from MA-EBOV infection. Treatment of 90 mg/kg starting 1 day post-infection protected 50% of mice.	N/A
	Apilimod[123]	Prevents trafficking of incoming virus to NPC1/membrane fusion sites	IC_{50} 0.01–0.14 μM[c,d,k,i]	N/A	N/A

Targeted step of virus lifecycle	Small Molecule	MOA[a]	Efficacy *in vitro*	Efficacy *in vivo*	Clinical Trial[b]
	Genistein[128]	Unknown	>70% inhibition at 100 μM[d,u]	N/A	N/A
	Tyrphostin AG1478[128]	Unknown	96–99% inhibition at 100|jM[d,u]	N/A	N/A
Virus Egress	5539–0062[132]	Inhibits EBOV VP40:tsg101 interaction	>90% inhibition at 100 μM[d,r]	N/A	N/A
	Compound 1 and derivatives[133]	Inhibits VP40:Nedd4 interaction	Compound 1: 5-fold decrease at 20 μM[u,z] Compound 4: at 1 μM, 100-fold[u,z] and 10-fold[u,r] decrease	N/A	N/A

[a] Mechanism of Action

[b] Phase and NCT identifier of clinical trial and status information from ClinicalTrials.gov

[c] MARV

[d] EBOV

[e] SUDV

[f] Hela cells

[g] Intramuscular route

[h] HFF-1, HMVEC-TERT cells

[i] Primary macrophages and HUH7 cells

[j] Intravenous

[k] Vero/Vero E6 cells

[l] Oral route

[m] EBOV minigenome assay

[n] MARV minigenome assay

[o] HepG2 cells

[p] BSR T7/5 cells

Curr Opin Virol. Author manuscript; available in PMC 2020 April 16.

[q] Fluorescent polarization assay for the interaction of NP and NPBP

[r] EBOV VLP

[s] EBOV GP pseudotyped virions

[t] MARV GP pseudotyped virions

[u] 293T/293FT cells

[v] Intraperitoneal route

[w] SUDV, TAFV, RESTV and BDBV GP pseudotyped virions

[x] HUVEC cells

[y] EAhy cells

[z] MARV VLP

[aa] Mouse-adapted EBOV

[*] estimated from figure