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REVIEW

Ebola virus outbreak, updates on current therapeutic strategies

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SUMMARY

Filoviruses are enveloped negative-sense single-stranded RNA viruses, which include Ebola and Marburg viruses, known to cause hemorrhagic fever in humans with a case fatality of up to 90%. There have been several Ebola virus outbreaks since the first outbreak in the Democratic Republic of Congo in 1976 of which, the recent 2013–2015 epidemic in Guinea , Liberia, and Sierra Leone is the largest in recorded history. Within a few months of the start of the outbreak in December 2013, thousands of infected cases were reported with a significant number of deaths. As of March 2015, according to the Centers for Disease Control and Prevention, there have been nearly 25 000 suspected cases, with 15 000 confirmed by laboratory testing, and over 10 000 deaths. The large number of cases and the high mortality rate, combined with the lack of effective Food and Drug Administration-approved treatments, necessitate the development of potent and safe therapeutic measures to combat the current and future outbreaks. Since the beginning of the outbreak, there have been considerable efforts to develop and characterize protective measures including vaccines and antiviral small molecules, and some have proven effective *in vitro* and in animal models. Most recently, a cocktail of monoclonal antibodies has been shown to be highly effective in protecting non-human primates from Ebola virus infection. In this review, we will discuss what is known about the nature of the virus, phylogenetic classification, genomic organization and replication, disease transmission, and viral entry and highlight the current approaches and efforts, in the development of therapeutics, to control the outbreak. Copyright © 2015 John Wiley & Sons, Ltd.

INTRODUCTION

Ebola virus (EBOV) and Marburg virus (MARV) are enveloped RNA viruses that belong to the family *Filoviridae* and appear, under the electron microscope, as thread-like or filamentous [1]. The genus *Ebolavirus* includes five EBOVs, of which EBOV is the causative agent of the current outbreak [2]. Three Ebolaviruses, including EBOV, cause Ebola virus disease (EVD) that is clinically characterized by a severe hemorrhagic fever in humans with a fatality rate of up to 90% [3,4]. In the 1970s, the first three EBOV outbreaks were indigenous to the Democratic Republic of Congo (formerly known as Zaire) and Sudan with some cases identified in other African countries, but since then, no additional cases were identified until late 1994 [5]. In 2013–2015, the largest outbreak of EBOV hemorrhagic fever started in West Africa [6,7]. The epidemic started in Republic of Guinea, initially in the prefecture of Guéckédou on December 2013 [8]. Few cases were discovered soon after in Sierra Leone, Liberia, and Nigeria and one case in Senegal [7]. In the 2013–2015 outbreak, at least 22 859 were identified as suspected cases and 9162 as confirmed deaths according to the World Health Organization (WHO) as of this writing [7]. Several studies have

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Abbreviations used
EBOV, Ebola virus; MARV, Marburg virus; EVD, Ebola virus disease; VSV, vesicular stomatitis virus; BDBV, Bundibugyo virus; SUDV, Sudan virus; TAFV, Taï Forest ebolavirus; RESTV, Reston ebolavirus; NP, nucleoprotein; GP, glycoprotein; VLPs, virus-like particles; KPN-α, karyopherin-α; HF, hemorrhagic fever; DIC, disseminated intravascular coagulation; DCs, dendritic cells; TIM-1, T-cell immunoglobulin and mucin domain protein; NPC1, Niemann–Pick C1; LDL, low density lipoprotein cholesterol; CatL, cathepsin L; CatB, cathepsin B; RhAPC, recombinant human activated protein C; rNAPc2, recombinant nematode anticoagulant protein c2; siRNAs, small interfering RNAs; WHO, World Health Organization.

considered different strategies to stop EBOV infection *in vitro* and *in vivo* such as the development of antiviral small molecules [9–17], antisense technology [18,19], and monoclonal antibody cocktails (such as ZMapp) [20]. In addition, selective estrogen receptor modulators, for example, clomiphene and toremifene [21], ion channel blockers, for example, verapamil and amiodarone [22], and adenovirus, vesicular stomatitis (VSV) and human parainfluenza-based vaccines as well as vaccines based on virus-like particle preparations have demonstrated potential efficacy [23,24]. In the current review, we will describe the structure of the virus, the nature of the disease, and the current advances in the development of therapeutics.

Taxonomy and morphology

Ebola virus and MARV belong to the *Filoviridae* family of enveloped viruses, order *Mononegavirales*, and are characterized by having a filamentous morphology [1,4]. The filoviruses are known to be the major causes of hemorrhagic fever in humans and non-human primates [25]. The filamentous morphology of the viruses led to the name Filoviridae, which is derived from the Latin word "*filum,*" which means filament [1]. The genus *Ebolavirus* includes five species: *Bundibugyo ebolavirus* (BDBV), *Sudan ebolavirus* (SUDV), *Taï Forest ebolavirus* (TAFV; previously known as *Cote d'Ivoire ebolavirus*), *Reston virus* (RESTV) and EBOV (formerly known as the Zaire ebolavirus; Figure 1) [2]. The SUDV and EBOV appear to be more involved with the known outbreaks and are more
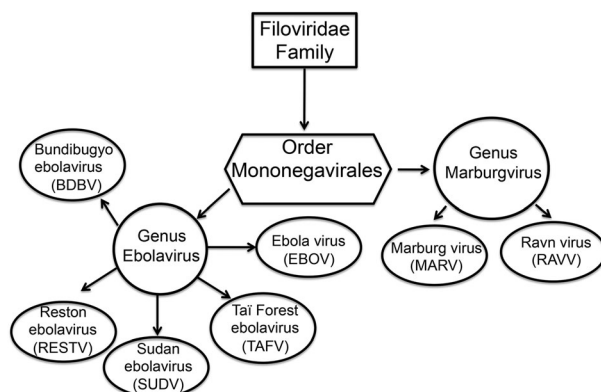

Figure 1. Taxonomic classification of Ebola virus (EBOV) and Marburg virus (MARV). EBOV formerly known as Zaire Ebola virus is one of five ebolaviruses belonging to the genus *Ebolavirus*, while MARV is one of the two viruses belonging to the genus *Marburgvirus*

pathogenic than the RESTV and the TAFV [26]. Of the known EVD-causing viruses, EBOV is considered to be the most dangerous and was involved in the largest number of outbreaks including the 2013–2015 outbreak [2,7]. The RESTV was a cause of fatal hemorrhagic disease in non-human primates with no reported involvement in human disease so far [27]. Based on genetic similarities, the Ebolaviruses are closely related to Marburgviruses [2].

Evolution and genetic variations

The viral glycoprotein (GP) gene sequence was the major determinant of the phylogenetic classification of filoviruses [28–31]. EBOV and MARV differ by approximately 55% at the genomic level and by up to 67% at the amino acid level [28–33]. A difference in the GP gene organization has been reported between EBOV and MARV [28–33]. Between the five known species of the genus *Ebolavirus*, there is a difference of 37–40% and 34–43% at the nucleotide and amino acid levels, respectively [28,32]. This indicates that BDBV, REBOV, SUDV, TAFV, and EBOV are distinct and represent different species. Despite the difference in the Ebolavirus species, they show some degree of genetic stability [28,32,34]. The genetic stasis suggests that there is an association with a particular host, and genetic variations are just ways of evolution and adaptation to the natural host. Within the order *Mononegavirales*, based on the extent of sequence homologies in the N-terminus of the RNA-dependent RNA polymerase, the filoviruses are considered more similar to paramyxoviruses than to rhabdoviruses [35].

Genome organization and replication

The filoviral genome consists of a non-segmented, negative single-stranded linear RNA molecule, which makes up 1.1% of the total virion mass. The genome size is approximately 19 kb for MARV and EBOV [36–38]. The viral genome is made up of seven genes arranged from the 3′ to the 5′ end in the order (i) nucleoprotein (NP), (ii) viral structural proteins VP35, and (iii) VP40, (iv) GP, (v) VP30, (vi) VP24, and (vii) RNA-dependent RNA polymerase gene (L) [4]. At the 3′ and 5′ ends of the genome, there are highly conserved non-coding nucleotide sequences, and they function as promoters in the transcription and replication of filoviruses and, in addition, as signals for packaging the viral genome

[38–41]. It is important to note that transcriptional initiation and termination are also controlled by highly conserved motifs flanking the genes of the filovirus [42,43]. The individual gene transcriptional signals begin at the 3′ end and terminate with a transcriptional termination (polyadenylation) site [36–38]. Transcription is terminated when the polymerase encounters a series of five to six uridines where the polymerase stops and adds a long poly-A tail to the transcripts [36–38]. The stability and level of transcripts are influenced by the long non-coding sequences at the 3′ and 5′ ends of the genome [37,38].

Ebola virus proteins

Filoviruses, including the EBOV, produce seven structural proteins encoded by the viral genome [43]. Four of the seven proteins (NP, VP30, VP35, and L) are known to directly bind to the negative-sense RNA genome and form the ribonucleoprotein complex [43]. The remaining three structural proteins include GP, the major membrane spike protein, VP40, and VP24 as the major and minor matrix proteins, respectively [44–46]. The VP40 and VP24 proteins interact with the viral envelope and the nucleocapsid serving as bridging molecules [44–46]. In addition, a non-structural soluble GP precursor protein is produced and subsequently cleaved into sGP and delta (Δ) peptide [47].

Recent studies show that the VP40 protein is capable of forming filamentous viral particles and, when coexpressed with the GP protein, the GP spikes localize to the surface of the virion, suggesting that both proteins control the virion shape and morphology [48]. The minor matrix protein, VP24, is unique to filoviruses and plays a role in budding and assembling, due to its association with lipid membranes [44]. Together with NP, VP24 enhanced the budding and releasing of VP40-induced virus-like particles (VLPs) and is required for assembly and formation of a functional ribonucleoprotein complex [49–53]. In addition, VP24 was described as a regulator of viral transcription and replication [54]. It was reported that VP24 binds karyopherin-α (KPN-α) and thus preventing the nuclear accumulation of tyrosine phosphorylated STAT1, which leads finally to inhibition of IFN signaling [55–57].

VP35 is the polymerase cofactor that associates with the EBOV RNA genome and acts as a Type I IFN antagonist [58,59]. Expression of EBOV VP35 revived the replication defective influenza virus, lacking the NS1 protein, whereas other EBOV proteins could not. Furthermore, VP35 inhibited IFN-β production triggered by poly I:C, mutant influenza virus infection, or by Sendai virus infection, suggesting that EBOV VP35 acts as an IFN antagonist quite similar to influenza protein NS1 [58–60]. It was reported that the EBOV VP35 inhibited interferon production through (i) binding and masking the EBOV dsRNA and (ii) inhibiting the phosphorylation of interferon regulatory factor (IRF)-3 by TBK-1/IKKε, which impairs IRF-3 localization to the nucleus [58–60]. In addition to its ability to block IFN production, VP35 showed an ability to counteract and block other antiviral responses in the target cell. VP35 interaction with the Small Ubiquitin-like Modifier (SUMO) E2 enzyme Ubc9 and the SUMO E3 ligase PIAS1 of the SUMOylation machinery facilitated the selective SUMOylation of IRF-7 and the blocking of the IFN transcription [60,61]. Other groups showed that VP35 inhibited the Protein Kinase R (PKR) negative effect on the host translational machinery probably by interfering with PKR activity and decreasing eIF2α phosphorylation [62,63]. Furthermore, VP35 showed other means of antagonizing the host cell antiviral responses by blocking the RNA silencing machinery [64].

Ebola virus GP gene, when transcribed and translated, produces two proteins. The first small polypeptide product of the GP gene is sGP [46]. The second and full-length larger gene product is the GP, which is produced by inserting an adenosine residue during transcription through RNA editing [28]. The GP is processed by furin enzyme, a subtilisin/kexin-like convertase localized in the trans-Golgi, at a polybasic cleavage site [65]. The mature protein has an N-terminus GP1 subunit (Mr 14 000) and a C-terminal GP2 subunit (Mr 26 000) linked by a disulfide bond [65]. The GP1 subunit contains several N-glycosylations and O-glycosylations [66]. The mature protein exists as homo-trimers on the surface of viral particles, and the trimerization is mediated by GP2 [46].

Ebola virus transmission and disease

Deadly infections with EBOV and Avian flu can be transmitted between animals and humans [67,68]. In humans, EBOV infections have typically occurred in rural settings, possibly through contact with infected non-human primates' body fluids [67]. Evidence strongly suggests fruit bats as the natural

reservoirs for EBOV [69]. In Africa, fruit bats such as *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata* were found as the natural hosts of the EBOV [4]. In addition, non-human primates, such as apes or monkeys, can be infected [70]. Bats dropping partially eaten fruits represent the likely means by which the virus transmission to humans starts. Mammals like gorillas, apes, monkeys, or duikers feeding on the partially eaten fruits can acquire the infection, which can be then be transmitted to humans [67]. In Guinea, it is believed that the current outbreak started when a child played with insectivorous bats from a colony of Angolan free-tailed bats near his or her village [71]. Early outbreaks in the Democratic Republic of Congo have usually involved bat reservoirs in or around a subterranean gold mine [71]. People who are in healthcare settings, in close contact with secretions of an infected patient, or involved in the burial of infected dead bodies are at a higher risk for EBOV infection [72]. Transmission of EBOV occurs through close contact with skin and secretions of an individual suffering from active infection. Urine, saliva, sweat, feces, vomitus, breast milk, and semen, and virus-contaminated objects can transmit the infection as well [73]. The infection with EBOV needs a relatively high viral dose of 10^{7}–10^{8} pfu/g [4]. Transmission is not likely to occur before the onset of symptoms. The incubation period of EBOV before the onset of symptoms ranges from 2 to 21 days [4]. The EBOV enters the body through small skin lesions and mucous membranes, after which it reaches the blood stream [73,74]. There is no evidence for aerosol transmission although it cannot be excluded [75].

Ebola virus infection quickly progresses to lethal hemorrhagic disease in humans and non-human primates [73]. The filoviral hemorrhagic fever (HF) is considered to be the most dangerous and severe form of all viral hemorrhagic fevers with intense clinical manifestations such as hemorrhage, coagulation disorders, shock, and hepatic failure [76]. The prodromal symptoms include chills, fever, headache, malaise, and myalgias. With progression of the disease, more severe and disease specific symptoms are experienced, such as gastrointestinal symptoms (vomiting, abdominal pain, and diarrhea), respiratory (cough, chest pain, and shortness of breath), vascular symptoms (conjunctival hemorrhage, postural hypotension, and extremity edema), and neurological symptoms characterized by severe headache, confusion, and coma [76].

Death from filoviral infections is usually the result of hypovolemic or septic shock as a consequence of several complications such as increased permeability of blood vessels, hypotension, coagulation problems, multi-organ failure, and focal tissue destruction [4,77]. Humoral response can be detected around Days 7–11 post-infection, which can influence survival or death of the patient [78].

Ebola virus infects a wide range of tissues including skin, mucous membranes, and internal organs. Of all the EBOV tissue effects, liver focal necrosis is the most prominent that leads to disseminated intravascular coagulation (DIC) [79]. Spleen and lymph nodes show extensive follicular necrosis and necrotic debris [80,81]. Infected lungs show interstitial edema and hemorrhage with clear alveolar damage. Additionally, the heart shows edema and focal necrosis [80,81]. The primary site of viral replication is believed to be monocytes, macrophages, and dendritic cells (DCs), and they are involved in the viral translocation to the lymph nodes through the lymphatics [82,83]. Endothelial dysfunction can lead to a wide range of vascular effects leading to hemorrhage and increase in vascular permeability [84].

Ebola virus glycoprotein structure and functional organization

Ebola virus GP is a viral class I membrane fusion protein that is quite similar to the prototypic HIV-1 envelope protein gp160 and influenza virus hemagglutinin in organization and function [85,86]. The GP1 subunit contains the receptor-binding domain and mediates interaction with the cognate receptor on the host cell, interacts with, and preserves the conformation of the GP2 transmembrane subunit [87]. The GP2 subunit contains the machinery required for the fusion of the viral membrane with the host cell membrane [85,86]. The GP1 subunit consists of three domains: (i) the N-terminus half is highly conserved and forms the base that interacts extensively with GP2 maintaining it in its prefusion state; (ii) the head that contains the receptor-binding sequences; and (iii) the C-terminus domain, which comprises highly glycosylated regions named the glycan cap and the mucin domain [88] (Figure 2). The GP folding is mediated by the glycan cap, and in addition, the glycan cap has been reported to have a crucial role in entry [89,90]. The mucin domain may not
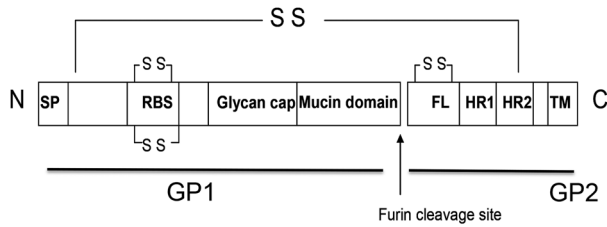
Figure 2. Structural organization and features of the Ebola virus (EBOV)–glycoprotein (GP). Linear diagram of EBOV-GP showing the cleavage of GP0 by the cellular Golgi endopeptidase furin into GP1 and GP2. SP, signal peptide; RBS, receptor-binding sequence; FL, GP2 fusion loop; HR1, GP2 N-terminal heptad repeat; HR2, GP2 C-terminal heptad repeat; TM, GP2 transmembrane domain. Lines and "SS" indicate intrasubunit and intersubunit disulfide bonds

be required for the EBOV-GP dependent pseudo-virus entry *in vitro* but is proposed to mediate viral adhesion to specific cell types and play a role in evasion of immune responses, by masking key neutralization epitopes [89,90]. The base and the glycan cap are connected by a short loop. The GP2 transmembrane fusion subunit contains an N-terminal hydrophobic internal fusion peptide in addition to the N-terminal and C-terminal helical heptad repeats (HR1 and HR2; Figure 2) [85,91]. The close interaction of the GP2-HR1 and GP2-HR2, triggered by GP processing during the viral entry, drives the fusion of the viral and host membranes [85,91].

Ebola virus entry into host cells

Ebola virus infects many cell types with a broad mammalian host cell tropism [92,93]. The broad host cell range suggests that the EBOV uses either a ubiquitously expressed receptor in all cell types or the virus can recognize and bind to several surface receptors expressed by different host cells. Evidence through several studies supports the latter scenario. The C-type lectins, for example, DC-SIGN and L-SIGN, which are highly expressed on the surface of many cell types, were shown to mediate the entry of EBOV and the entry of other viruses [94,95]. However, it is important to note that some cells that are permissive to the EBOV infection do not express the C-type lectins, and their role in natural infection remains to be determined. Moreover, several studies have shown that DC-SIGN/L-SIGN, LSECtin, hMGL, β1-integrins, and Tyro-3 family receptors may be involved in the attachment of EBOV to the host cell surface, but none of the cellular factors proved to be essential for viral entry [94,96–101].

The T-cell immunoglobulin and mucin domain protein (TIM-1), a T-cell costimulatory molecule and phosphatidylserine receptor, was identified as a candidate cell surface receptor for EBOV [102,103]. TIM-1 is highly expressed on human epithelial cells including airway epithelium that are known to be targets for EBOV [102]. The fact that other permissive cells, such as macrophages and DCs, do not express TIM-1 suggests that EBOV use a receptor other than TIM-1 to gain entry into those immune cells [102]. The role of TIM-1 in EBOV entry, *in vivo*, remains to be determined.

The host protein Niemann–Pick C1 (NPC1) was recently shown as an important receptor involved in EBOV virus entry [104,105]. NPC1 is largely expressed by all cells and is known to localize to late endosomes and lysosomes [106]. The lysosomal efflux of low-density lipoprotein cholesterol (LDL) is mediated in part by NPC1 [106]. A study showed that a small molecule inhibitor of NPC1 can block EBOV entry *in vitro* [105]. Furthermore, NPC1 deficient hamster cell lines are not permissive to EBOV [104]. These studies suggest that the requirement for NPC1 may be indispensable for EBOV entry. The NPC1 role in EBOV entry is completely distinct from its function in cholesterol metabolism [104]. A study showed that the cleaved but not the uncleaved EBOV-GP binds to late endosomes, and the binding is NPC1 dependent suggesting that priming of GP by cysteine proteases is required for binding to NPC1 [105]. The homotypic fusion and vacuole protein-sorting complex, which aids in the fusion of endosomes to lysosomes, might be involved in viral entry but is not essential [104].

Following attachment to the surface receptor, the virus is internalized via a macropinocytosis-like mechanism [107]. The process involves the formation of plasma membrane ruffles [108]. The large particle size of EBOV might suggest why it uses the macropinocytosis for internalization, but the use of this route is dependent on the GP interactions rather than the virion size. TIM-1 and other lectins have been reported to trigger macropinocytosis [102]. Studies have shown that EBOV-GP pseudotyped viruses colocalize with Rab7 in late endosomes and a dominant negative inhibitor of Rab7 reduces infection [104,107]. This suggests that delivery to late endosomes is crucial for viral entry. However, it is not yet known whether the virus delivers its genome to the cytoplasm directly from late

endosomes or whether other cellular compartments like lysosomes are involved in the entry process.

The role of caveolae in EBOV entry remains unclear, and further studies are needed to show whether caveolae are involved or not in the EBOV entry [109,110]. Clathrin-mediated endocytosis has been implicated in EBOV entry [111,112]. However, based on evidence from different studies, EBOV may use different pathways to gain entry into host cells depending on the cell type, viral isolate, and possibly the viral load.

Generally, EBOV-GP, as a class I viral GP, is primed for fusion by furin cleavage at a single site within the virus producer cell [113]. This cleavage exposes a peptide that triggers fusion between the viral and host cell membrane [113]. The cleavage of GP into GP1 and GP2 by furin is dispensable both *in vitro* and *in vivo* and if blocked does not affect the infectivity of the virus. This has led to the conclusion that the furin-mediated cleavage of GP protein in virus-producing cells does not drive fusion, which is indeed driven by host endosomal cysteine proteases in the target cell [114]. Both cathepsin B (CatB) and cathepsin L (CatL) cysteine proteases were identified as host proteases involved in EBOV-GP processing [115]. Structural and biochemical evidence suggests that the cleavage in the loop connecting the base and the head of the GP1 subunit exposes the underlying GP2 fusion loop allowing it to function in the fusion process [86,87]. It is possible that the cleavage of EBOV-GP by cysteine proteases unmasks the binding site for an unknown receptor, possibly NPC1, with a role in promoting fusion [86,87]. The higher binding of CatL treated EBOV-GP pseudotyped virions to target cells supports this speculation [116].

Current therapeutic approaches for the prevention of Ebola virus infection

The lack of pre-exposure and post-exposure therapeutic interventions against EBOV and the lethality of EBOV infections necessitate the development of antivirals and protective vaccines. Formalin-fixed or heat-inactivated virus-based vaccines were produced soon after the outbreak in 1976 to protect guinea pigs and non-human primates [84,117]. However, the protection reported in both studies was inconsistent, and the elicited immune response was insufficient to protect baboons against lethal doses of EBOV. Several studies have shown

tremendous efforts to develop subunit vaccines against EBOV since 1990. Earlier studies led to the conclusion that mice and guinea pigs are the most protected species, while non-human primates need to mount a more robust immune response to achieve protection [118–121]. However, a study published in Nature showed that DNA-based immunization and boosting with adenoviral vectors, which encode viral proteins, generated cellular and humoral immunity in cynomolgus macaques that remained asymptomatic for more than 6 months, with no detectable virus after the initial challenge, while the unvaccinated animals died in less than a week [122]. The controversy in the results obtained from vaccine studies on non-human primates indicates that more extensive research is required to develop vaccines that provide complete protection against EBOV.

In September 2014, cAd3-EBOV, an experimental vaccine against two EBOVs (EBOV and SUDV) jointly developed by GlaxoSmithKline and the National Institutes of Health, started a Phase I clinical trial and was administered to volunteers in Oxford, Bethesda, and Mali where the initial results were promising [123]. The cAd3-EBOV is derived from chimpanzee adenovirus type 3 and engineered to express the EBOV-GP and SUDV-GP. The second vaccine candidate, the rVSV-EBOV, was at first developed by the Public Health Agency of Canada and then by Merck Inc. [123,124]. The rVSV-EBOV was effective against EBOV in non-human primates [123,124]. Other vaccine candidates were developed by Johnson and Johnson Pharmaceuticals and by the Chinese government, and they have been approved for clinical trials. Being an acute infection, instantaneous protective measures including antiviral small molecules and passive antibody therapy may be promising in combating EBOV HF.

In the current outbreak, several promising treatments are being considered for development and currently or will soon undergo clinical trials. One of the considered treatments is the transfusion of whole blood or purified serum from EBOV survivors that was identified as a possible treatment in early 1970s [125,126]. In December 2014, the first clinical trial of this therapy, including 70 patients, started at the Eternal Love Winning Africa 2 treatment center in Liberia and is sponsored by the Gates Foundation in collaboration with national health authorities and WHO. Further clinical trials will soon start in Guinea

and Sierra Leone [123]. ZMapp, is a combination of three humanized murine monoclonal antibodies developed by Mapp Biopharmaceuticals Inc. and produced in tobacco plants. In mice, 43% of ZMapp-treated animals survived, and it proved to be highly effective in a trial involving rhesus macaque monkeys [127,128]. ZMapp has been used to treat some patients in the current West Africa outbreak; however, the efficacy remains to be determined [123].

A combination of small interfering RNAs (siRNAs) against the EBOV-L, VP24, and VP35 formulated in stable nucleic acid lipid particles showed protection in rhesus monkeys against lethal EBOV infection [18]. The drug was formulated using lipid nanoparticle technology by Tekmira Pharmaceuticals Corp and now named as TKM-Ebola. A phase I clinical trial involving healthy volunteers was initiated in 2013–2014 but was quickly suspended due to adverse side effects [129]. However, later the Food and Drug Administration approved the use of TKM-Ebola in emergency situations. Several small molecule inhibitors of viral replication or entry were developed by different groups. Of the EBOV antivirals, favipiravir, also known as T-705 or Avigan, chemically defined as a pyrazinecarboxamide derivative and initially developed in Japan to treat influenza infection, is effective against a mouse model of EBOV infection [130]. Favipiravir showed inhibitory activity against influenza virus, West Nile virus, yellow fever virus, and foot and mouth disease virus in addition to flaviviruses, arenaviruses, bunyaviruses, and alphaviruses [131]. Activity against enteroviruses and Rift Valley virus has also been documented [131,132]. Favipiravir inhibits viral RNA-dependent RNA polymerase [133]. The adenosine nucleoside analog, BCX4430, is a broad-spectrum antiviral drug initially developed by BioCryst Pharmaceuticals against hepatitis C and is currently tested against EBOV by the United States Army Medical Research Institute of Infectious Diseases [134–136]. BCX4430 shows efficacy against a wide range of other viruses, including bunyaviruses, arenaviruses, paramyxoviruses, coronaviruses, and flaviviruses [134–136]. BCX4430 protects against EBOV and MARV in both rodents and monkeys, even when administered up to 48 h post-infection [134–136]. Brincidofovir, is another antiviral drug with activity against EBOV as well as cytomegalovirus, adenovirus, and smallpox *in vitro* and in animal models [137].

A set of new compounds, such as FGI-103, FGI-104, FGI-106, dUY11, and LJ-001, has been developed against filoviruses including EBOV and a variety of newly developed drugs that have the potential to target the EBOV VP35 and VP40 [138–141]. Drugs currently approved for other diseases inhibited EBOV *in vitro* and in animal models, for example, cationic amphiphiles (amiodarone, dronedarone, verapamil, clomiphene, and toremifene), Na^+ channel and Na^+/K^+ exchange blockers (amiloride), Na^+/K^+ ATPase inhibitors (digoxin, digitoxin, and ouabain) [21,22,142]. A small molecule, 1E7-03, targeting the host cellular protein phosphatase 1, which controls EBOV VP30 dephosphorylation, inhibited EBOV replication *in vitro* [143]. AVI-7537, a phosphorodiamidate morpholino oligomer targeting the EBOV VP24 RNA transcript, is undergoing phase I clinical trial [144]. In addition, novel broad-spectrum antiviral small molecules that inhibited the entry of a wide range of viruses, including EBOV, by blocking CatL have been described in [16]. Other interventions include (i) recombinant human activated protein C (RhAPC) for the post-exposure treatment of EBOV infection in rhesus macaques and (ii) recombinant nematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of tissue factor initiated blood coagulation, which prolonged survival time in rhesus macaques challenged with EBOV lethal dose [145,146]. Previously described efforts demonstrate that an anti-EBOV drug is within reach, and the EBOV epidemic may be under control in the near future (Figure 3).

CONCLUDING REMARKS

The recent outbreak that started in Guinea highlighted the importance of the development of preventive and therapeutic interventions to protect against any future outbreaks. The high fatality of the EBOV infection in addition to the existence of bats as the primary animal reservoirs and non-human primates, as intermediate carriers for the virus, make disease prevention and treatment a complex process [3,4,69,70]. Several studies have reported the development of passive immunotherapies in the form of antibodies and small molecule antivirals with inhibitory activities against EBOV infection *in vitro* and in animal models of infection [125–144]. A considerable number of the developed antivirals are currently being tested for efficacy and safety in clinical trials, which suggests that potent

Figure 3. Different therapeutic approaches to prevent Ebola virus (EBOV) infection. The therapeutic approaches include (i) vaccines, for example, cAd3-EBOV and rVSV-EBOV; (ii) passive antibody therapy, for example, immune serum transfusion and ZMapp antibodies; (iii) different antiviral molecules targeting (a) viral RNA transcripts, for example, TKM-Ebola small interfering RNAs (siRNAs) and the AVI-7535 morpholino oligomer targeting the VP24 RNA transcript, (b) different viral proteins, (c) host proteases, such as cathepsin L and B, or (d) host phosphatases, such as IE7-03 targeting protein phosphatase 1 (PP1)

anti-EBOV drugs are within reach in the near future [123,129,144].

CONFLICT OF INTEREST
The authors have no competing interest.

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