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Therapeutics for postexposure treatment of Ebola virus infection

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Abstract

The current Ebola virus disease (EVD) outbreak in West Africa is the largest with over 5100 deaths in four West African countries as of 14 November 2014. EVD has high case-fatality rates but no licensed treatment or vaccine is yet available. Several vaccine candidates that protected nonhuman primates are not yet available for clinical use. Slow development of vaccine-stimulated immunity, sporadic nature and fast progression of EVD underlines the need for the development of effective postexposure therapeutic drugs. WHO encouraged the use of untested drugs for EVD to curb the fast-spreading outbreak. Here, we summarize therapeutics for EVD including monoclonal antibody-based therapy and inhibitors of viral replication including our recently developed small-molecule inhibitors of VP30 dephosphorylation.

Keywords

1E7-03; antibody therapy; Ebola virus; postexposure drugs

The current Ebola virus disease (EVD) outbreak in West Africa is spreading faster than efforts to control it. As of 14 November 2014, this is the largest outbreak where over 5100 people in four West-African countries have died since the outbreak began in February 2014. EVD is a severe, fatal illness in humans. EVD outbreaks have a case fatality rate of 52.3% for EVD by all Ebola viruses; 52.6% for EVD caused only by Zaire Ebola virus (EBOV) and 47% for the current EVD outbreak in West Africa [1,2]. Infection with Ebola virus can cause death to susceptible hosts within 5–10 days after the appearance of symptoms. The

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virus spreads in the human population by contact with infected bodily fluids [3]. Initial flu-like clinical signs can progress to internal bleeding, diarrhea and vomiting that can lead to organ failure and death. Severely ill patients require intensive supportive care. Several promising vaccine candidates have been found to protect nonhuman primates (NHPs), but none have been approved for clinical use [4–8]. However, the slow development of vaccine-stimulated immunity (at least 3 weeks), and the sporadic nature and fast progression of EVD underline the need for the development of effective postexposure therapeutic drugs. On 12 August 2014, the WHO published the statement that it was ethical to use untested drugs to treat patients infected with Ebola virus in light of the scale of the outbreak and high number of deaths. In this review, we summarize the current progress in the development of the potential postexposure drugs. Ebola virus belongs to the mononegavirales family Filoviridae, which includes three genera: *Ebolavirus*, *Marburgvirus* and *Cuevavirus* [9]. The genus *Ebolavirus* has five members: Bundibugyo virus (BDBV), Zaire Ebola virus (EBOV), Reston virus (RESTV), Sudan virus (SUDV) and Taï Forest virus (TAFV) [9]. Like all mononegavirales family viruses, Ebola virus has a nonsegmented, negative-strand RNA genome and produces enveloped virions [10]. EVD is caused by BDBV, EBOV, SUDV and TAFV, while RESTV is not pathogenic for humans [10]. Marburg virus (MARV) is another virus that belongs to the family Filoviridae [9] and also causes hemorrhagic fever similar to EVD. Ebola virus genome encodes eight proteins, which mediate the entry, replication and egress of the virus from the host cell [11]. The development of therapeutics for postexposure treatment of EVD has targeted the viral proteins as well as host proteins and pathways. We have described here several groups of postexposure anti-Ebola virus drugs including monoclonal antibody (mAb)-based therapy; inhibitors of viral entry, transcription and replication, and inhibitors of viral budding and egress (Figure 1). We also have discussed our novel small molecule, 1E7-03, that inhibited phosphorylation of VP30 protein that is involved in the activation of viral transcription.

Passive antibodies treatment

Antibody therapy became a popular treatment strategy against infectious pathogens in the late 19th and early 20th centuries, but later it was largely replaced by antibiotics and vaccines. High-dose intravenous immunoglobulins have been used to treat certain viral infections in immunocompromised patients (e.g., cytomegalovirus, parvovirus B19 and enterovirus infections) [12]. In viral disease, antibodies block viral entry into uninfected cells, promote antibody-directed cell-mediated cytotoxicity by natural killer cells and neutralize the virus alone or with the participation of complement [12]. Hyperimmune serum from EBOV-infected goats showed protection against EBOV infection in mice and guinea pigs when administered within 24 h postexposure [13]. The goat immunoglobulins were also tested in human volunteers and administered to several workers suspected of being infected with EBOV [13]. Success of these tests warranted the approval of the goat immunoglobulins as emergency treatment for EBOV infection in the Russian Federation [13]. Hyperimmune equine immunoglobulins were prepared from EBOV-infected horses and protected four out of five baboons infected with EBOV [13]. However, in the follow-up study, hyperimmune equine immunoglobulins only showed a delay in the onset of EVD in monkeys [14], thus rising significant skepticism for their effectiveness. This skepticism was further

substantiated by the inability of neutralizing human mAbs, KZ52, to protect against EBOV infection in rhesus macaques when the antibodies were administered intravenously 1 day prior to the lethal EBOV injection [15]. The EBOV glycoprotein (GP) is the only known target for neutralizing antibodies, and EBOV neutralizing as well as non-neutralizing antibodies were found in the serum of convalescent patients and experimentally infected NHPs [16,17]. In contrast to these earlier studies, more recent multiple independent studies have indicated that passively administered antibodies can provide effective postexposure therapy in NHPs after infection with the otherwise lethal doses of EBOV or MARV (Table 1). Administration of polyclonal IgG antibody from survivors in NHPs at 48 h after infection with either MARV or EBOV protected two-thirds of animals [18]. A third of animals developed mild and delayed signs of disease followed by full recovery [18]. This study clearly demonstrates that postexposure antibody treatments can protect NHPs and opens an avenue for filovirus therapies for humans using established US FDA-approved polyclonal or mAb technologies. However, taking in account the high case-fatality (lethality) rates and low number of survivors, treatment with polyclonal antibodies from survivors in humans is very limited. Thus for the postexposure therapy with antibodies, the development of recombinant mAbs is necessary. The anti-EBOV mAbs protected rodents against EBOV exposure, but did not have protective effects in NHPs against lethal exposure [19,20]. In 2012, two different groups demonstrated that combination of mAbs partly protected lethally infected rhesus macaques when administrated 24 h postexposure [19,21]. Plant cells (*Nicotiana benthamiana*) were used by one of these groups to produce MB-003 mAb cocktail [21]. About two-thirds of NHPs that received plant produced MB-003 24–48 h postinfection survived [21]. The mAbs were detected in blood of surviving animals along with the virus-induced IgGs [19]. In contrast, mAbs were not detected in blood of the animals that succumbed to infection [19]. EBOV-GP-specific humoral and cell-mediated immune response was also induced in the surviving animals. Also another group reported complete protection from EVD in cynomolgus macaques with different combination of three mAbs directed against EBOV envelope GP [22]. US Mapp Biopharmaceutical, Inc. (CA, USA), together with Defyrus, Inc. (ON, Canada), licensed an antibodies cocktail produced in tobacco plants under the name of ZMapp. ZMapp rescued 100% of rhesus macaques when treatment was initiated up to 5 days postexposure [23]. ZMapp has not yet been tested in humans for safety or effectiveness. During the current EVD outbreak, seven patients received an experimental treatment with ZMapp. Five of them survived EVD. Because the case-fatality rate of current EVD outbreak in West Africa is about 50%, it is difficult to make a definite conclusion about efficacy of ZMapp treatment in humans [24].

Recently administration of mAbs along with adenovirus-vectored IFN- α in NHPs extended the treatment window to 3 days post-exposure when an early viremia and symptoms were already detectable [25]. A total of 75% of cynomolgus macaques and 100% of rhesus macaques survived when treatment was initiated after the detection of viremia at 3 days postinfection.

Currently mAb-based therapies are the most efficient at reversing the progression of a lethal EBOV infection in NHPs [26]. Generally, polyclonal neutralizing antibodies are more efficient for treatment of viral infection than mAbs. However, the lack of the systems for

production of polyclonal antibodies against EBOV mandated the mAb production. However, filoviruses have high flexibility and variability to evade neutralizing antibody treatment [27]. Although recent studies have demonstrated that antibody therapy is a promising approach for the treatment of filovirus infections, it is still unclear if the combination of mAbs with or without cytokines will be efficient to combat EVD in humans.

Inhibition of viral entry

Inhibition of viral entry is a common approach for the development of postexposure antiviral drugs (Table 2). There are two major ways for prevention of viral entry: modification of viral proteins involved in the initiation of infection and inhibition of cell receptors for virus attachment. Filovirus GP is the only protein involved in initiation of infection. GPs are heavily glycosylated proteins and contain high-mannose residues. Several groups of antiviral compounds target envelope GPs including ‘carbohydrate-binding agents’ such as lectins and nonpeptidic antibiotics such as pradimicin A and S and benanomycin A [28]. Naturally produced lectins inhibit Ebola virus infection *in vitro* and thus have a potential future as antivirals. Human mannose-binding lectin (MBL), an endogenous C-type lectin, recognizes glycan structures, such as mannose, glucose and fucose, which may be exposed on the surface of Ebola virus particles. MBL targets diverse microorganisms for phagocytosis and complement-mediated lysis by binding to the surface glycans. As a result of common genetic variants, MBL serum levels in humans range from 0 to 10,000 ng/ml. 30% of the human population have MBL levels <500 ng/ml, which are associated with increased susceptibility to infections [29]. MBL binds to EBOV and MARV viruses resulting in blocking virus interaction with DC-SIGN and induction of complement-mediated virus neutralization [30]. Physiological doses of MBL rescued about 40% of mice from lethal injection when administered post-Ebola virus exposure [31]. Commercial-grade recombinant human MBL is provided by Enzon Pharmaceuticals. Unfortunately, MBL has a complex quaternary structure unsuitable for large-scale cost-effective production. A less-complex chimeric fusion lectin L-FCN/MBL76 with similar ligand recognition and enhanced effector functions was developed [32]. L-FCN/MBL76 reduced infection of cell culture by EBOV significantly greater than the other lectins. Further investigations have to be done to demonstrate the efficiency of MBL treatment in humans.

Most nonhuman lectins induce a strong immune response in human and not suitable for EVD therapy. Griffithsin (GRFT) is a red-algae-derived lectin that binds the terminal mannose residues of N-linked glycans on the surface of EBOV [33]. GRFT displays no human T-cell mitogenic activity and, unlike many other lectins, does not induce production of proinflammatory cytokines in treated human peripheral blood mononuclear cells [33]. Daily subcutaneous doses of GRFT demonstrated a minimal toxicity in rodents of two different species [33]. Further investigations have to be done to study its efficacy in the inhibition of Ebola virus infection.

Because GP is the only filovirus protein involved in initiation of infection, it has been intensely studied for its ability to bind cellular receptors [34,35]. Filoviruses use a complex route of cell entry that depends on numerous cellular receptors and factors [35–37]. Thus the inhibition of a single receptor may not be sufficient for prevention of Ebola virus infection.

In vitro screens of FDA-approved drugs that can prevent filovirus entry identified amiodarone (a multi-ion channel inhibitor and adreno receptor antagonist); amiodarone-related agent dronedarone; and the L-type calcium channel blocker verapamil. All these drugs were potent inhibitors of filovirus cell entry at concentrations that are routinely reached in human serum during antiarrhythmic therapy (Table 3) [38]. The exact mechanism of Ebola-virus entry inhibition by amiodarone, dronedarone and verapamil is not known. The advantage of this approach is that FDA-approved drugs are immediately available and can be quickly repurposed for treatment of filovirus infections.

Multiple cellular receptors utilized by Ebola virus for entry are signaling downstream through the phosphorylation mediated by overlapping sets of kinases. Thus, inhibition of a downstream kinase may provide a universal approach comparing with the inhibition of diverse cellular receptors used for virus attachment and entry. Ebola virus infection of macrophages and dendritic cells induced MAPK signaling pathway [39]. Pyridinyl imidazole-based MAPK inhibitors efficiently block EBOV replication in cultured macrophages and primary dendritic cells differentiated from monocytes by blocking the viral entry [39].

Following attachment, virions enter the cells by endocytosis. Acidification of the endocytic vesicles and the fusion of virus and host membranes result in the release of the nucleocapsid (NC) into cytoplasm [40]. Small-molecule inhibitor benzylpiperazine adamantane diamide-derived compound inhibited GP binding to the endosomal membrane protein Niemann–Pick C1, and prevents release of the NC into the cytoplasm. It is a potential candidate for antiviral therapy [41].

Taken together, several FDA-approved drugs were found to block EBOV entrance [38]. They are available for immediate use, but the efficacy of each single drug or their combination against Ebola virus infection in humans is not known.

Inhibition of viral transcription & replication

Following filovirus entry, transcription of negative-strand RNA is the first viral process in the cell. A common approach for inhibition of viral RNA synthesis is the use of synthetic nucleoside analogs (Table 2). Novel synthetic adenosine analog, BCX4430 developed by North Carolina based company BioCryst Pharmaceuticals, inhibits filoviral infection in human cells and completely protects NHPs from MARV virus infection when administered 48 h after infection [42,43]. BCX4430 inhibits viral RNA polymerase function, acting as a nonobligate RNA-chain terminator.

The pyrazinecarboxamide derivative T-705 (favipiravir) was developed in 2002 by Toyama Chemicals (Japan) as an inhibitor of influenza A virus replication, and is currently in late-stage clinical development for the treatment of influenza A virus [44]. T-705 is converted by host enzymes to ribofuranosyl-5'-triphosphate that acts as a nucleotide analog selectively inhibiting the viral RNA-dependent RNA polymerase or causing lethal mutagenesis upon incorporation into the virus RNA [45,46]. Administration of T-705 starting 6-days postlethal exposure of EBOV in mouse model induced rapid virus clearance, reduced biochemical parameters of disease severity and prevented a lethal outcome in 100% of the animals [47].

T-705 has the additional advantage that it can be used for oral administration, in contrast to many other drugs that require injections. Most nucleoside analogs are associated with tissue-specific toxicities, such as peripheral neuropathy, myopathy, nephropathy, and pancreatitis and lactic acidosis with hepatic steatosis [48]. In contrast, T-705 demonstrated its beneficial antiviral effects without significant toxicity to the host [47].

Another approach to inhibit viral RNA synthesis is formation of inhibitory complexes with single-stranded DNA analogs (phosphorodiamidate morpholino oligomers [PMOs]) or double-stranded small interfering RNA (siRNA) (Table 2). They both recognize target viral RNA sites with high specificity and affinity. Effective target sites in viral RNA are the highly conserved sequence regions important either in the preinitiation or initiation of translation, or in long-range RNA–RNA interactions involved in viral RNA synthesis [49]. Several Ebola virus proteins (L, VP24 and VP35) have been used as targets in these approaches. Viral RNA-dependent RNA polymerase (RdRp [L protein]) is necessary for all aspects of viral-RNA synthesis, ranging from genome synthesis to mRNA synthesis, capping and polyadenylation [50]. VP35 is a multifunctional double-stranded RNA (dsRNA) binding protein that plays important roles in viral mRNA synthesis and replication of negative-sense RNA viral genome [51]. VP35, nucleoprotein (NP) and VP24 are essential components for NC formation [52]. Antisense-PMOs are nuclease-resistant and water-soluble single-stranded DNA analogs that can enter cells and form stable duplex with complementary RNA [49]. PMOs are easy to synthesize and they can be administered in the isotonic delivery vehicles [53]. Recently, a combination of two PMOs (AVI-6002) consisting of AVI-7537 targeting the VP24 gene and AVI-7539 targeting VP35 has been developed and optimized by Sarepta Therapeutics under contract with US Department of Defense. Administration of AVI-6002 in NHPs after injection of a lethal dose of EBOV led to 60% survival [54]. AVI-6002 is now progressing into the late-stage clinical development as the optimal therapeutic candidate [54]. Because PMOs are sequence-specific DNAs, the efficacy of therapeutics depends on the existence of the conserved targeting sequences and can be offset by the emergence of the resistant mutants. This problem can be overcome by the use of several targeting sequences against conserved viral sites or the site important for the interaction with host proteins, and also by mutated PMOs that may compensate for the predicted base-pair mismatches [53].

siRNAs targeting various Ebola virus sequences were also used as potential therapeutics. The liposome-formulated siRNAs targeting EBOV RNA polymerase L fully protected guinea pigs against lethal EBOV exposure when administered shortly postinfection [55]. Lipid-formulated combination of three siRNAs developed by Tekmira Pharmaceuticals (British Columbia, Canada) under the name of TKM-Ebola targeting L polymerase, VP24 and VP35 proteins protected two out of three rhesus monkeys when four postexposure treatments were administered and protected 100% animals when seven postexposure treatments were administered [56].

Aptamers are short RNA or single-stranded DNA oligonucleotides that have high specificity and affinity for their targets. Two aptamers that bind VP35 protein with high affinity and specificity have been delineated [51]. These aptamers can compete with dsRNA for binding to VP35, disrupting VP35–NP interaction and inhibiting EBOV polymerase complex [51].

Also small-molecule inhibitors of VP35–NP interaction have been reported [57]. NMR mapping experiments and high-resolution x-ray crystal structures showed that selected small molecules bind to VP35 interferon inhibitory domain (IID) that is important for replication complex formation by interacting with the viral NP. They inhibited VP35–NP interactions *in vitro* and during EBOV replication [57].

Recent screening of a library of more than 2000 diverse compounds identified a potential small-molecule inhibitor of RdRp indoline alkaloid type compound CMLDBU3402. It inhibited EBOV RNA transcription and viral gene expression in cell culture model [58].

All Ebola-virus inhibitors described above are specific to viral targets, thus the efficacy of therapies can be limited by the emergence of resistant mutants.

Recently, we published a novel approach targeting host–viral proteins interaction for the development of Ebola virus drugs [59]. The ribonucleoprotein complex of Ebola virus includes VP30 protein in addition to NP, VP35 and the polymerase (L) [60]. The polymerase complex can mediate both the transcription of individual genes and replication of the whole genome. While the exact mechanism of the polymerase switch between the transcription and replication modes is unknown, several studies pointed to VP30 as a transcription activation factor unique for filoviruses [61–63]. EBOV VP30 protein is phosphorylated at two serine clusters at positions 29–31 and 42–46 and at a threonine in position 52, located close to the RNA-binding domain [64]. Phosphorylation of VP30 blocks the ability of the viral polymerase to function during transcription, but not genome replication [64–66]. Our study showed that PP1 controls VP30 phosphorylation *in vivo* [59]. The PP1 holoenzyme consists of a constant catalytic subunit (PP1 α , PP1 β/δ or PP1 γ) and a variable regulatory subunit that determines the localization, activity and substrate specificity of the phosphatase [67]. PP1 catalytic subunit associates with a regulatory subunit through one or a combination of short binding motifs, such as the RVxF motif [68]. Engagements of different combinations of these motifs define both the composition of PP1 holoenzymes and their unique specificity for different substrates in various cell compartments. We recently developed a library of PP1-targeting small molecules that were modeled to fit the RVxF-accommodating cavity of PP1 [69]. Our virtual screening of 300,000 compounds identified a tetrahydroquinoline derivative, 1E7-03 [70], that was devoid of toxicity, and displayed a half-life greater than 8 h when administered to mice. 1E7-03 bound to PP1 *in vitro* and prevented shuttling of PP1 into the nucleus [70]. 1E7-03 increased EBOV VP30 phosphorylation and effectively suppressed replication of EBOV particles in cultured cells [59]. Analysis of the effect of 1E7-03 on EBOV transcription and replication using the mini-genome system showed reduction of EBOV transcription, but not replication. These results identified novel small-molecule inhibitor of EBOV transcription and pointed to PP1 as novel therapeutics target. Future experiments will demonstrate if 1E7-03 protects animals exposed to lethal doses of EBOV.

Inhibition of budding & egress

Inhibition of virus budding and egress prevents virus dissemination, and small-molecule compounds that block budding could potentially block disease progression and transmission.

The Ebola virus matrix protein VP40 mediates the plasma membrane binding and budding of the virus prior to egress [71]. It is localized under the lipid envelope of the virus where it bridges the viral lipid envelope and NC. The VP40 is the most abundantly expressed protein of the virus. A number of studies have demonstrated that specific deletions or mutations of VP40 abrogate viral egress [72,73]. The VP40 of EBOV contains viral PPxY late budding domains (L domains) that interacts with cellular proteins including Nedd4E3 ubiquitin ligase [74]. Because L domains of EBOV are important for budding and are highly conserved in a wide array of RNA viruses, they represent potential broad-spectrum targets for the development of antiviral drugs [75]. A small-molecule compound that inhibited Nedd4-PPxY interaction and PPxY-dependent budding was found in *in silico* screening (Table 2) [75].

Other approaches

Inhibitors of host proteins that play an important role in viral infection are also under development. Host cellular endoplasmic reticulum α -glucosidases I and II are required for the maturation of viral envelope proteins [76]. Inhibition of these enzymes prevents the correct folding of viral GPs and leads to their degradation. Imino sugars, which inhibit α -glucosidases, are approved for the treatment of Type-II diabetes and Type 1 Gaucher's disease. These drugs include Zavesca (miglustat or NBDNJ), Precose (acarbose) and Lyset (miglitol). The CM-10-18 and its derivatives which inhibit imino sugar α -glucosidase protected over 70% of mice from lethal exposure to MARV and EBOV when administered at 4 h postexposure and the administration continued for 10 days (Table 3) [76,77].

Deregulation of proinflammatory cytokine production by macrophages, which are primary sites of filovirus replication, plays a major role in the immunopathogenesis of EVD. The filoviruses have mechanisms for inhibiting type I IFN production and response [78]. Despite this, EVD is associated with robust IFN- α production in humans and macaques [77,79]. However, little IFN- β (or IFN- γ) is produced in response to EBOV infection. Early postexposure treatment with IFN- β significantly increased survival time of rhesus macaques infected with a lethal dose of EBOV and MARV [80].

High-throughput screening of small molecular inhibitors of Ebola virus *in vitro* is a widely used approach. It often results in the discovery of molecules with unknown mechanisms of inhibition. One such inhibitor is an antioxidant compound NSC 62914. The compound was found to exhibit antifilovirus activity in cell-based assays and protected mice following exposure with EBOV and MARV (Table 2) [81]. Another small-molecule compound found in *in vitro* screening is diazachrysene, which displays broad-spectrum inhibition activity against lethal viral hemorrhagic fever pathogens, including Ebola virus, Rift Valley and dengue viruses 1–4 (Table 2) [82]. A single intraperitoneal dose of this compound administered 24 h postinfection was sufficient to completely protect mice against a lethal injection with mouse-adapted strains of EBOV and MARV [83]. Its analogs were also highly efficacious against EBOV and MARV infection with little or no associated cellular toxicity [84].

In vitro screen of FDA- and ex-US-approved drugs with antiviral activity against Ebola virus identified a set of selective estrogen receptor modulators (SERMs), including clomiphene and toremifene, which act as potent inhibitors of Ebola virus infection (Table 3) [85]. Treatment of EBOV infected mice protected about 50% animals when treated with toremifene and about 90% animals when treated with clomiphene. The anti-EBOV activity of SERMs occurred even in the absence of estrogen receptors, suggesting that SERMs might utilize a different form of estrogen receptor-based mechanism of inhibition.

Conclusion

Development of drugs against Ebola virus shares the common fate with number of other rare infectious diseases. Ebola virus was discovered in 1976, but until the occurrence of the current outbreak, the overall fatality was less than 1200 people. Thus it was unpredictable whether there would ever be a need for large-scale treatment. This low number of potential infections has not attracted financial interests of pharmaceutical companies or private investors. Thus, the lack of FDA-approved drugs for EVD treatment is not only a scientific but also a financial problem. Overall, there are little or no human safety data available for drugs described in this review. For several of them safety and efficacy were demonstrated in NHP model, but the relationship between NHP model and filoviral infections in humans still needs to be evaluated. Even for the most developed and licensed drugs such as BCX4430 (BioCryst Pharmaceuticals, NC, USA), TKM-Ebola (Tekmira Pharmaceuticals, British Columbia, Canada) and AVI-7537 (Sarepta, MA, USA), the Phase-I trials were on hold by FDA until recently. Most drugs described in this review have only been tested in mouse models and their efficacy in the NHP model still needs to be evaluated. There is a separate group of FDA-approved drugs, which were evaluated for safety in humans, but their efficacy for EVD treatment is still not well established. Over and above drug candidates have shown protection when administered shortly after infection. However, most of them had no therapeutic benefits beyond the time window of 2 days postinfection. The therapeutic window to treat EVD disease is still very narrow highlighting the necessity to develop strategies for clinical management of symptomatic EVD beyond supportive therapy [47]. Only T-705 and ZMapp demonstrated therapeutic benefits when treatment was initiated 5–6 days post-EBOV exposure and fully prevented death (100%) in animals. The WHO decision to use yet untested drugs for EVD treatment poses a significant risk. Except for the FDA-approved drugs that can be redirected for EBOV treatment, there is no other drug supply available for immediate use. Moreover, the negative results from experimental treatment can both increase health problems and reduce the public enthusiasm thus hindering future development of EVD cure.

Future perspective

The continued EBOV outbreak increased federal and private funding for preclinical and clinical trials of the existing vaccines and antivirals. This increase in funding is likely to result in the development of novel antifeloviral therapeutics in the near future. There is a possibility that Ebola virus infection will become endemic in West Africa, thus requiring concerted efforts for its eradication. The increased interest in filoviral infection will also be likely to lead to increased basic science efforts to understand the biology of the virus and

may provide future avenues for antiviral drug and vaccine development. Thus, we will likely see an increase in the involvement of leading researchers in filoviral studies and fast progress in the understanding of filovirus biology.

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EXECUTIVE SUMMARY

Passive antibodies treatment

- Postexposure administration of polyclonal antibodies and monoclonal antibodies cocktail (ZMapp) demonstrated promising results in nonhuman primates.

Inhibition of viral entry

- Antiviral compounds that target viral glycoprotein were developed while multiplicity of the identified viral receptors makes their targeting difficult. Targeting endosomal lipid transporter, Niemann-Pick C1, led to EBOV inhibition. Number of US FDA-approved drugs block Zaire Ebola virus (EBOV) entry and may be used for further drug development.

Inhibition of viral transcription & replication

- Inhibitors of RNA synthesis include RNA-polymerase inhibitors, BCX4430 and T-705, AVI-6002 phosphorodiamidate morpholino oligomers, and TKM-Ebola siRNAs. VP35 was targeted with aptamers and small molecules that inhibited EBOV. VP30 phosphorylation was increased by PP1-targeting 1E7-03 compound that also inhibited EBOV.

Other approaches

- VP40 deletions or prevention of its interaction with Nedd4E3 ubiquitine ligase prevented viral budding. Inhibition of correct viral glycoprotein folding by Type II diabetes FDA-approved drugs inhibited EBOV and Marburg virus infection. Other FDA-approved drugs effective against EBOV include IFN- β treatment and SERMs. Broad-spectrum inhibitors include antioxidant compound NSC62914 and diazachrysene.

Figure 1. Potential Zaire Ebola virus therapeutic interventions

The EBOV life cycle is shown in bold. Potential drugs are shown in blue rectangles. Red blunt arrows show the stages of virus life cycle that inhibited by drugs.

Ab: Antibody; BAD: Benzylpiperazine adamantane diamide; EBOV: Zaire Ebola virus; GRFT: Gri thsin, red-algae-derived lectin; MBL: Mannose-binding lectin; NC: Nuclear capsid; PI: Pyridinyl imidazole; PMO: Phosphorodiamidate morpholino oligomer.

Table 1

Antibody-based treatments.

Name	Target	Comments	Ref.
Polyclonal IgG from survivors NHP	N/A	Protect NHP when administered 46 h postinfection with EBOV and MARV	[18]
Human neutralizing mAbs	GP	Single mAbs KZ52 specific for EBOV protected rodents but not NHP	[15]
Combination of two neutralizing mAbs	GP	Human–mouse chimeric mAbs partly protected NHP after administration 24–48 h postinfection	[19,20]
Combination of three neutralizing mAbs MB-003	GP	Mouse-human chimeric mAbs developed in tobacco plant partly protected NHP after administration 24–48 h postinfection	[21]
Combination of three neutralizing mAbs (ZMab)	GP	Complete survival of NHP when three doses of mouse mAbs were administered 3 days apart beginning at 24 h after a lethal injection with EBOV	[22]
Combination of mAb and IFN- α	GP	75% cynomolgus and 100% of rhesus macaques survived when treated at 3 days postinfection	[25]
Optimized combination of mAbs (ZMapp)	GP	Rescue 100% of rhesus macaques when treatment is initiated up to 5 days postexposure	[23]

EBOV: Zaire Ebola virus; IgG: Immunoglobulin G; mAb: Monoclonal antibody; MARV: Marburg virus; N/A: Not available; NHP: Nonhuman primate.

Table 2

Small-molecule and experimental inhibitors of Zaire Ebola virus and Marburg virus.

Name	Target	Comments	Ref.
MBL	GP	Postinfection treatment protects 40% mice from lethal injection with EBOV	[31]
Commercial-grade MBL	GP	Chimeric fusion protein L-FCN/MBL76 reduced infection of cells by EBOV	[32]
GRFT	Glycan structures	GRFT binds N-linked glycans on the surface of EBOV. Activity against EBOV is not known	[33]
Pyridinyl imidazole	MAPK P38	Partly blocks entrance of EBOV in macrophages and dendritic cells	[39]
Benzylpiperazine adamantane diamide-derived compound	Endosomal membrane protein Niemann-Pick C1	Blocks release of nucleocapsid from the endosomes in cell culture	[41]
BCX4430	RNA polymerase	Protects NHP from MARV; inhibits viral RNA polymerase function, acting as a nonobligate RNA-chain terminator	[42,43]
T-705 (favipiravir)	RNA polymerase	Acts as a nucleotide analog. Treatment at day 6 postinfection protects 100% of mice	[47]
AVI-6002 (PMO)	VP24 and VP35	Postinfection treatment protects 60% NHP from lethal injection with EBOV	[54]
CMLDBU3402	RNA polymerase	Small-molecule EBOV RNA transcription inhibitor	[58]
TKM-Ebola	RNA polymerase VP24 and VP35	Combination of siRNAs in lipid particles protects guinea pigs and monkeys	[55,56]
Aptamers	VP35	Aptamers binding VP35 protein disrupts its interaction with NP	[51]
Small-molecule inhibitors	VP35	Inhibited VP35–NP interactions and EBOV replication	[57]
1E7-03	Protein phosphatase-1 and VP30	Increased Vp30 phosphorylation and inhibited EBOV transcription and replication	[59]
Small-molecule inhibitor of VP40	VP40	Inhibited Nedd4–PPxY interaction and PPxY-dependent budding	[75]
CM-10-18 Imino-sugar	α -glucosidase	Postinfection treatment protects 70% mice from lethal injections with EBOV and MARV	[76]
NSC 62914 antioxidant	Unknown	Protects in cell-based assays and in mice after exposure to EBOV and MARV	[81]
Diazachrysene and its derivatives	Unknown	Broad-spectrum inhibition of EBOV, Rift Valley and Dengue Fever viruses, in cell-based assay. Protects 100% mice after exposure to EBOV and MARV	[82–84]

EBOV: Zaire Ebola virus; GRFT: Griffithsin, red-algae-derived lectin; MARV: Marburg virus; MBL: Mannose-binding lectin; NHP: Nonhuman primate; NP: Nucleoprotein; PMO: Phosphorodiamidate morpholino oligomer.

Table 3

US FDA-approved drugs that potentially inhibit Zaire Ebola virus.

Name	Target/disease	Comments	Ref.
Amiodarone	Multi-ion channel inhibitor and adreno receptor antagonist	Block viral entry at concentrations used for antiarrhythmic therapy	[38]
Dronedarone	Multi-ion channel inhibitor and adreno receptor antagonist	Block viral entry at concentrations used for antiarrhythmic therapy	[38]
Verapamil	L-type calcium channel inhibitor	Block viral entry at concentrations used for antiarrhythmic therapy	[38]
Imino sugars: miglustat, acarbose, miglitol	α -glucosidase	Postinfection treatment protects 70% mice from lethal injections with EBOV and MARV	[76,77]
IFN- β	Immune disorders	Increases time of survival in NHP after lethal EBOV exposure	[80]
Toremifene	Estrogen receptor modulators	Protects 50% of mice from lethal exposure with EBOV	[85]
Clomiphene	Estrogen receptor modulators	Protects 90% of mice from lethal exposure with EBOV	[85]

EBOV: Zaire Ebola virus; MARV: Marburg virus.