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Short review

ADVANCES IN TACKLING FILOVIRUSES

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Filoviruses are virulent pathogens that cause deadly haemorrhagic fever in humans and non-human primates. There is currently no approved drug or vaccine to tackle this disease. Two vaccine platforms that use adenovirus vectors have completed phase I studies, while a recombinant vesicular stomatitis virus-based vaccine has successfully completed a phase III trial. Intricate macromolecular therapeutics have also been developed, most notably those based on antibodies or interfering RNA or RNA-surrogates. Most small molecules active against filoviruses have not yet advanced to clinical trials, except favipiravir, which was proven to be safe, and GS-5734, which has entered trials.

Key words: ebola; Marburg; hemorrhagic fever; vaccines; small molecules

INTRODUCTION

Filoviruses (lat. *Filoviridae*) are a family of viruses belonging to the *Mononegavirales* order [1]. Most of the members of this family cause fatal hemorrhagic fever in humans and non-human primates (NHPs) [2].

The Filoviridae family is comprised of three genera – *Marburgvirus* (discovered in 1967 [3] and named after the city of Marburg in Germany), *Ebolavirus* (discovered in 1976 [4, 5] and named after the Ebola river in the Democratic Republic of the Congo) and *Cuevavirus* (discovered in 2009 [6] in Spain and named after the Spanish word for 'cave'). The former two genera have caused several outbreaks among humans in the last 50 years, resulting in at least 2.885 reported cases and 1.972 deaths [7, 8]. More recently, Ebola virus caused the first-ever filovirus epidemic of an unprecedented scale in West Africa, from 2013 to 2016, which alone accounted for at least 28.616 cases and 11.310 deaths, according to the World Health Organization [9].

There are no FDA approved drugs or vaccines to tackle this deadly virus. In this paper, we provide a review of the most promising therapeutic candidates that have been developed to date.

VACCINES

There have been several attempts to generate vaccines against filoviruses, but only three have made it into advanced phases of clinical trials. These will be described here in more detail.

Scientists from the Public Health Agency of Canada (PHAC) developed the rVSV-ZEBOV vaccine in the early 2000s [10]. As the name suggests, the *Zaire ebolavirus* (ZEBOV) glycoprotein (GP) gene was inserted into the genome of the recombinant vesicular stomatitis virus (rVSV), a replication-competent viral vector (Figure 1). The GP is the only filoviral protein expressed on the surface of the virion, and is therefore immunologically important. The vaccine was initially proven to be safe and effective in rodents, such as mice, hamsters and guinea pigs [11–13]. Afterwards, NHP trials showed that the vaccine provided full protection when administered from 31 to 7 days prior to infection, and partial protection when administered from 3 days before to 1 day after infection [14–17].

However, several phase I studies that were performed in 2014 and 2015 showed that, overall, 22% of subjects had fever after vaccination, and other adverse effects (AEs) were noted [19, 20]. Fi-

nally, a cluster-randomized phase III study was performed during the West African epidemic in the Conakry region of Guinea, and Tomkolili, and Bombali regions of Sierra Leone on a large scale [21]. A ring vaccination approach inspired by the strategy that resulted in smallpox eradication was utilized. Lists of contacts (and contacts of contacts) of infected patients were followed and archived, after which randomized 1:1 clusters were either immediately vaccinated or vaccinated with a 21 day delay. There were no cases of Ebola registered within 10 days of vaccination in the immediately vaccinated clusters (2119 people in total), while 16 cases were registered in the delayed vaccination clusters (2041 people in total). More than half (53.9%, 3149 people) of patients reported some AEs, but only 80 reported a serious AE.

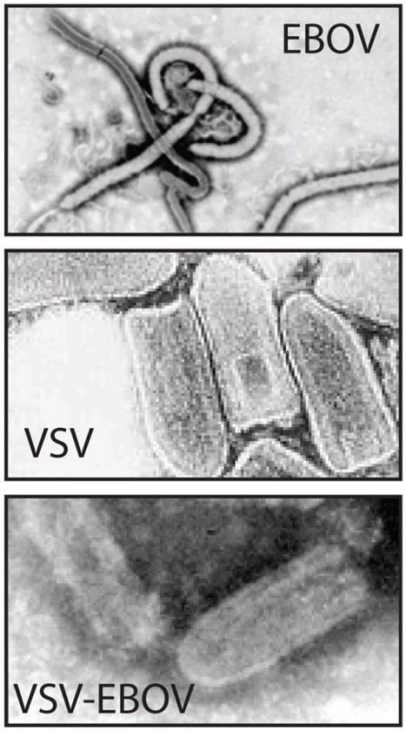


Figure 1. Transmission electron microscopy images of EBOV, VSV and rVSV-EBOV [18]

The other two advanced vaccines both utilize an adenovirus (AdV) vector. In these vaccines, the EBOV GP occupies the native adenovirus early region, furnishing a nonreplicating virus [18].

The first of the two was initially developed in the early 2000s by the National Institute of Allergy and Infectious Diseases (NIAID), beginning with a plain DNA vaccine [22]. The NIAID effort was later joined by GlaxoSmithKline. Due to safety reasons, initial experiments involved modified EBOV GP delivered by DNA vaccination, before moving on to full-length wild-type glycoprotein, which was proved to be safe in a phase I study [23]. After these results, a chimpanzee adenovirus 3 (cAd3) vector-based vaccine was evaluated [24]. The vaccine encoded both the *Zaire* and the *Sudan* species' wild-type GPs. In the NHP trials, it was, however, observed that an additional boost with an attenuated vaccine of a poxvirus (modified vaccinia Ankara, MVA) vector was needed for a longer lasting protection of 10 months after vaccination. Finally, cAd3-EBO phase I studies were initiated in 2014, with both Zaire GP, or a combination of Sudan and Zaire GPs, respectively [25, 26]. Glycoprotein-specific antibodies were induced in a dose-dependent manner in all participants, with the doses ranging from 1×10^{10} viral particle units (pu) to 2×10^{11} pu. The AEs were dose-dependent, with up to 56% of volunteers developing very mild AEs, and up to 20% developing fever, which luckily did not last more than one day. In general, there were no serious AEs. Depending on the vaccine and on the dosage used, an additional MVA boost may or may not be required for protection lasting up to 48 weeks, as evidenced from antibody titers.

Lastly, the third group of vaccine platforms utilized a human AdV vector, and specifically, that of the Ad26 serotype, which is rarer than the common Ad5, and therefore more probable to override pre-existing immunity [27–31]. Two phase I studies have been conducted, where generally Ad26-ZEBOV was used for priming, and MV-BN-Filo was used as a boosting component, or vice versa. The boosting was performed 2, 4 or 8 weeks after the prime dose. The doses used were 5×10^{10} pu for priming and 1×10^{8} pu for boosting. Volunteers were observed for up to one year (360 days), the longest of all studies, and it was concluded that the regimen is safe, providing long lasting protection with an MVA-BN-Filo prime and subsequent Ad26-EBOV boost. The reverse order was better for a more rapid immunization, *i.e.* in emergency situations.

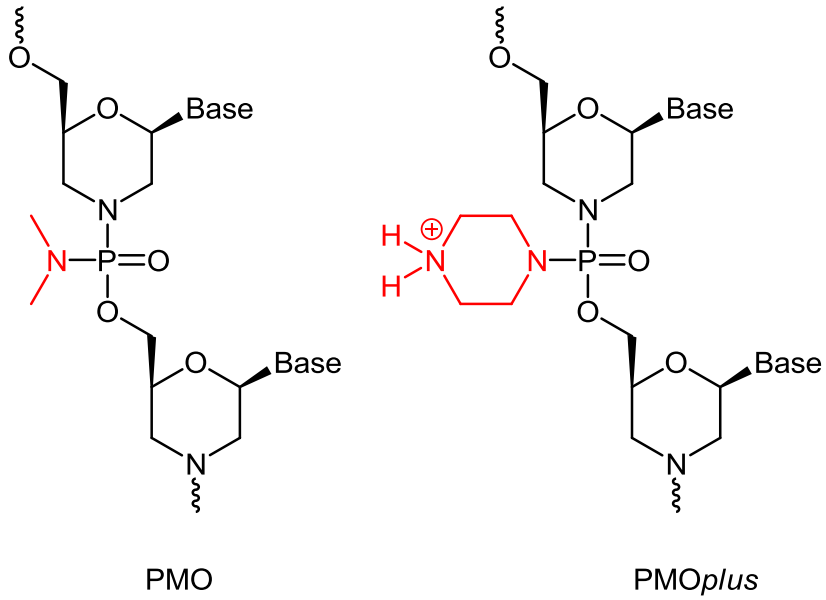
MACROMOLECULES IN EBOV THERAPY

Four approaches stand out in the field of potential macromolecular therapeutics.

The small interfering RNA (siRNA) approach was among the first to be developed. Namely, the idea was to synthesize siRNA complementary to the messenger RNA (mRNA) that encodes viral proteins, and then deliver it to an infected cell. The siRNA will target the mRNA, and after a complex of the two is formed, the cell will recognize it as abnormal, and the mRNA will be degraded, thus preventing translation, and resulting in silencing of

the viral genes. A Canadian company developed small lipid nanoparticles that carried siRNA complementary to mRNA of EBOV L polymerase, the membrane-associated protein (VP24), and the polymerase complex protein (VP35), a complex called TKM-Ebola [32–35]. The product, which was delivered intravenously, faced difficult phase I trials during the West African epidemic, with the FDA halting the study throughout 2014 and 2015 due to safety concerns [36]. Unfortunately, in a small phase II study in Sierra Leone, the formulation was not shown to improve survival when compared to historic controls [35].

Similarly, antisense phosphorodiamidate oligomers (PMOs, Scheme 1) were invented as early as the late 1990s in order to behave as RNA surrogates, and thereby target specific RNA sequences [37]. In the early 2000s it was discovered that therapy utilizing PMOs which mimic the EBOV VP24, VP35, and RNA polymerase L sequences can protect rhesus macaques [38]. Subsequently, a private company developed formulations such as AVI 6002, AVI 6003, AVI 7537 and AVI 7228, which targeted different Ebola or Marburg genes using the PMO technology, or, more specifically, a slightly different PMO*plus* approach (Scheme 1). These reached phases I studies, where they were proven to be well tolerated and safe, with potential as post-exposure prophylaxis for filovirus infections [39, 40]. However, to the best of our knowledge, no further development has been reported [41].



Scheme 1. Structure of PMO and PMOplus based oligomers [41]

A different approach was developed by PHAC, the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), and NIAID, and was later improved upon by US-based companies [42–45]. Specifically, a cocktail of three antibodies, called ZMapp, was derived from mice infected with Ebola. More precisely, the DNA that encodes monoclonal antibodies was collected from hybridomas and produced by combining immortal myeloma cancer cell lines with splenocytes of infected mice. The DNA was modified by genetic engineering to produce antibodies suitable for humans, and the appropriate genes were delivered to tobacco plants through an *Agrobacterium* which infects the plants. Finally, a large number of antibodies was collected from the dying plant.

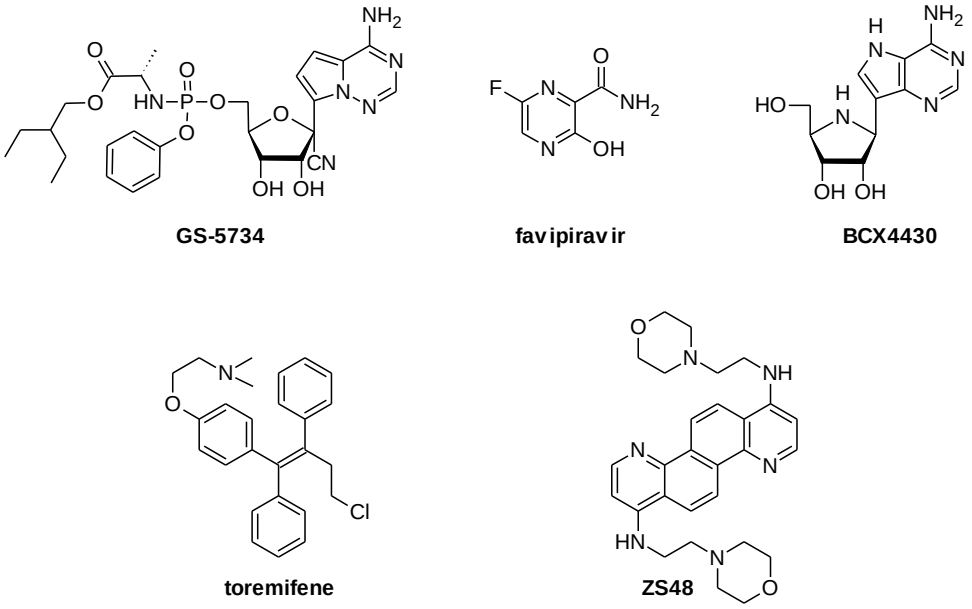
After the formulation was proven to be safe and effective in NHPs, clinical studies were initiated [42]. The fear of uncontrolled spread of the West African epidemic led to a clinical trial in infected humans [43]. In a group of controls that received only standard care at the time, 13 out of 35 patients (37%) died, compared to only 8 out of 36 patients (22%) who received standard care plus ZMapp. Thus, it was proven that the addition of ZMapp treatment to standard care would improve survival was 91.2%.

Finally, an approach utilizing glycodendritic structures that inhibit viral entry has been in development since the early 2000s [46]. Namely, the interaction of EBOV GP with a dendritic cell specific ICAM-3 grabbing non-integrin (DC-SIGN), which

facilitates viral entry into dendritic cells, can be inhibited by carbohydrates mimicking the glycans present in the viral GP. A multivalent presentation of carbohydrates is needed for effective interaction with lectins, therefore dendritic polymers with carbohydrate termini were developed; the most effective possess fullerene or even virus like particle (VLP) cores encompassing as many as 1620 copies of mannose at the surface [47, 48]. T-cells, which cannot otherwise be infected with EBOV, can be rendered susceptible by the addition of DC-SIGN, and these cells were used for proof-of-concept assays. Recombinant VSV expressing EBOV GP was used to mimic the virus, instead of the much harder to work with wild-type EBOV. In the assays, the dendrite polycarbohydrates showed IC_{50} values as low as ~900 pM.

SMALL MOLECULES

Since the late 1990s, a number of known and even FDA approved drugs, as well as newly synthesized small molecules, have been found to exhibit anti-EBOV activity. Above all, small molecules carry an advantage over vaccines and antibodies, in principle, being cheaper to produce. Moreover, depending on their mechanism of action, their activity may not be affected by mutations in the viral RNA. Scheme 2 outlines the most effective molecules, although many others have been, and are, being developed.



Scheme 2. Select examples of small molecules with anti-EBOV activity

Favipiravir, or T-705, is an antiviral drug developed by a Japan-based company. In essence, the compound is metabolized to a ribofuranosyl 5′-triphosphate derivative, which then enters the pathway of viral metabolism and inhibits the RNA-dependent RNA polymerase [49]. Even though it's *in vitro* activity is weak (IC_{50} = 67 μM), its toxicity is exceptionally low (CC_{50} > 1000 μM) [50]. Therefore, *in vivo* assays freely extended to doses as high as 300 mg/kg, and testing in mice proved that the compound is effective at doses as low as 30 mg/kg (under certain dosage regimes). A clinical trial was set up during the West African epidemic but performing a randomized study where one group of patients would receive standard care, while another would receive an additional experimental drug, was deemed inappropriate. Therefore, all patients received favipiravir. For the 99 patients that were treated, favipiravir was well tolerated and showed a mean decrease of viral load = 0.33 $\log_{10}$ copies/ml per day – in individuals that survived [51].

Another group of drugs, developed by USAMRIID, NIAID, and US-based private companies includes compounds such as BCX4430 and GS-5734. The drugs, which are nucleoside analogs, were reported to have a stronger affinity for viral RNA-polymerase than for that of native, host-cell (human) RNA-polymerase. These compounds, therefore, in a way similar to favipiravir, inhibit the RNA-related steps in the viral multiplication pathway. BCX4430 was discovered via a large scale high-throughput screening campaign [52]. It provided an IC_{50} value of ~3.4 μM, and it was the first small molecule to be tested in a NHP model, where

it cured 100% of monkeys with a 15 mg/kg dose, administered 2 times daily for 12 days. Under *in vitro* conditions, GS-5734, which was developed after BCX4430, proved to be superior, with an IC_{50} as low as 0.06 μM [53]. Most probably, its triphosphate metabolite is an RNA-chain terminator. The compound is not active in mice, but it did cure 100% of infected cynomolgus macaques with a 3.3 mg/kg daily dose. GS-5734 was given under compassionate use to two Ebola patients, and has entered clinical trials [54].

Lastly, an important group of EBOV inhibitors belongs to the class of compounds known as cationic amphiphilic drugs (CADs) [55, 56]. These molecules have a basic nitrogen atom separated by a flexible linker from a hydrophobic, most often aromatic, molecular core. The mode of action of these compounds is not fully elucidated, but it is clear that it is host-based, and that viral entry is being inhibited in the late endosome/lysosome stage. It is also clear these compounds increase Ca^{2+} levels within endosomes/lysosomes, while also lowering sphingosine levels and causing cholesterol accumulation [57, 58]. Toremifene, a selective estrogen receptor modulator, stands out among CADs, and its estrogen receptor related activity was proven to be irrelevant for anti-EBOV activity. Its IC_{50} value ranges from 0.97 to 1.73 μM, and it is able to cure 50% of mice with a 60 mg/kg/day dose. Our own research led to the development of diazachrysene derivatives, such as ZS48. These compounds have IC_{50} values as low as 0.34 μM, and can cure up to 90% of mice, notably with a low dose of 10 mg/kg, delivered intraperitoneally once daily for seven days [59, 60].

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НАПРЕДОК ВО БОРБАТА ПРОТИВ ФИЛОВИРУСИ

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Филовирусите се вирулентни патогени кои предизвикуваат смртоносна хеморагична треска кај луѓето и нехумани примати. Засега не постои одобрен лек или вакцина за борба против оваа болест. Две платформи на вакцини што користат аденовируси ги имаат поминато испитувањата од фазата I, додека вакцината заснована на везикуларен стоматитен вирус успешно ја има поминто фазата III од испитувањата. Развиени се и сложени макромолекулски терапевтски средства, најпознатото од нив е засновано на антитела или на интерферентна RNA или на сурогати RNA. Најголем дел од малите молекули активни против филовирусите сè уште не се дојдени до фаза на клинички истражувања, освен фавипиравир, за кој е докажано дека е безбеден, и GS-5734, кој е влезен во фазата на истражувања.

Клучни зборови: ебола; Марбург; хеморагична треска; вакцини; мали молекули