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Clinical Evaluation of Ebola Virus Disease Therapeutics

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Abstract

Ebola virus disease (EVD) was first described over 40 years ago, but no treatment has been approved for humans. The 2013–2016 EVD outbreak in West Africa has expedited the clinical evaluation of several candidate therapeutics that act through different mechanisms, but with mixed results. Nevertheless, these studies are important because the accumulation of clinical data and valuable experience in conducting efficacy trials under emergency circumstances will lead to better implementation of similar studies in the future. Here, we summarize the results of EVD clinical trials, focus on the discussion of factors that may have potentially impeded the effectiveness of existing candidate therapeutics, and highlight considerations that may help meet the challenges ahead in the quest to develop clinically-approved drug(s).

Keywords

Ebola virus; therapeutics; small molecule inhibitor; convalescent plasma; monoclonal antibody

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Ebola Virus Disease Therapeutics Are Urgently Needed

The outbreak of Ebola virus (EBOV) in West Africa from December 2013 to March 2016 was the largest ever reported to date, with 28,616 cases and 11,310 deaths (http://apps.who.int/iris/bitstream/10665/208883/1/ebolasitrep_10Jun2016_eng.pdf?ua=1).

EBOV belongs to the genus *Ebolavirus*, which causes EBOV disease (EVD) – clinically manifested by a spectrum of symptoms including fever, fatigue, muscle pain, vomiting, diarrhea, anorexia, rash, bleeding and multi-organ failure [1, 2]. Disease fatality rate can be up to 90% (<http://www.who.int/mediacentre/factsheets/fs103/en/>). The re-emergence of EBOV in the future cannot be ruled out because it can cause sporadic infections from unknown natural **reservoirs** (see Glossary) and potential transmission from EVD survivors, such as those that shed virus through bodily fluids including semen and breast milk [3, 4]. Due to high fatality rates, poorly-defined natural reservoirs and transmission mechanisms, in addition to the potential for weaponization, EBOV constitutes a major public health concern.

EBOV pathogenesis is currently only partially understood. EBOV is known to evade the Type I interferon (IFN) response through viral proteins VP30, VP35 and VP24 [5, 6], which contribute to initial viral replication and pathogenicity. Studies in non-human primates (NHPs) showed that early cellular targets of EBOV comprise macrophages and dendritic cells [7], which are currently recognized as two key players in pathogenesis [8, 9]. Dendritic cell maturation can be suppressed by EBOV, as evidenced by the failure of these cells to secrete proinflammatory cytokines and by the absence of upregulated co-stimulatory molecules, leading to impairment in antigen presentation to T-cells [10, 11]. Indeed, dysfunctional macrophages and dendritic cells likely cause deregulated innate immunity through the excessive production of proinflammatory cytokines and chemokines, as well as by suppression of adaptive immune responses against EBOV due to compromised presentation of antigen to lymphocytes and inadequate expression of co-stimulatory factors [12]. While there have been studies in mice [13] and humans [14] showing substantial involvement of adaptive immunity in advanced EVD, particularly the activation CD8⁺ T-cells, the systemic dissemination and robust viral replication stemming from an inability to control the infection at early disease stages eventually leads to multi-organ failure [1]. Therefore, strategies to develop targeted therapies against EVD are mostly focused on blocking viral entry and inhibiting viral replication [15].

Substantial efforts have been devoted to the development of EVD therapeutics in animal models over the past two decades, but remain untested in humans. The outbreak in West Africa greatly expedited the clinical evaluation of several promising therapeutics. Current candidate therapeutics mainly fall into 2 major categories: i) small molecule inhibitors, including licensed drugs to be repurposed for EVD treatment and newly developed nucleic acid-based products; and ii) immune-based therapeutics, including IFNs, plasma transfusion and monoclonal antibodies (mAbs). Full, or interim results of clinical trials for a number of experimental therapeutics have recently been reported. In this review, we summarize findings from those clinical trials that have been completed (Table 1, Key Table) and discuss the limitations that need to be overcome for the successful development of EVD-targeting therapies.

Small Molecule Inhibitors: Direct Intracellular Inhibition of EBOV

A popular approach in the search for effective therapeutics is the identification and characterization of **small molecules** that might inhibit EBOV, presumably through different mechanisms, including suppression of viral transcription and replication. Many small molecules, such as brincidofovir, BCX4430, favipiravir, GS-5734 and AVI-6002, have been shown to be protective in cultured cells, or in animal models such as mice and NHPs [15, 16], but remain to be assessed against EVD in humans. Recently, several small molecule drugs licensed for the treatment of other viral diseases, such as influenza and yellow fever [17, 18], or that have been newly developed against EVD, have been evaluated for their efficacy and effectiveness against EBOV infection in non-randomized clinical trials. These drugs include small compounds such as nucleotide analogs and small interfering RNAs (**siRNAs**) that target specific EBOV viral proteins.

Nucleotide Analogs: The Potential of Favipiravir in EVD Patients with Low Viral Loads

Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), or T-705, is a pyrazine derivative discovered from a screen of chemical compounds against influenza virus A/PR/8/34 (H1N1); it is modified intracellularly to form a purine nucleotide analog with inhibitory activity against viral RNA-dependent RNA polymerase (RdRP), but exhibits low or no inhibition of canine DNA and RNA polymerase, or human DNA polymerase [19, 20]. Favipiravir has shown inhibitory effects against a wide range of RNA viruses [17, 18, 20–25]. Recent studies in mouse models demonstrated post-exposure protection against EBOV through oral administration of favipiravir [26, 27]. In addition, favipiravir was shown to be well-tolerated in healthy or ill adults with uncomplicated influenza in phase 1–3 clinical trials [28].

In mid-November 2014, favipiravir was given to 39 patients with severe EVD admitted to the Sierra Leone-China Friendship Hospital [29]. Patients (17–39 years old) received oral favipiravir at doses of 800 mg bid on day 1, and 600 mg bid on day 2, based on recommendations for use in influenza infections [29]. Patients also received **supportive treatments** in the following days until recovery, hospital transfer or death. Survival rate and viremia in the favipiravir cohort were compared to control patients who were admitted to the same center earlier and treated with only supportive treatments. Results from the subsets with all endpoint observations available from the 2 groups (n=17 for the favipiravir group, n=18 for the control group) showed a higher survival rate in the favipiravir group (64.8% vs. 27.8%) [29]. Improvement of disease symptoms was observable in the favipiravir group, combined with a significant reduction in viral RNA load (>100 fold) determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR). These results indicated that favipiravir might be able to confer a survival benefit to EVD in humans [29].

In December 2014, a non-randomized, **single-arm** proof-of-concept clinical trial (the JIKI trial) was conducted to evaluate the safety and effectiveness of favipiravir at 4 treatment centers in Guinea ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02329054) identifier: NCT02329054) [28]. Among the EVD patients, 111 patients (99 aged 13 and older, 12 aged 6 and younger) received no other experimental therapies and completed the trial, and were thus included in the final analyses [28]. The **primary outcome** (Box 1) was mortality within a period of 14 days. Doses in

adults were determined based on results from mouse studies [30], as well as on pharmacokinetic simulation and dosage tests in humans [28].

Adult patients were given oral favipiravir at doses of 2400 mg, 2400 mg and 1200 mg every 8 h on day 0, and 1200 mg bid for the following 9 days (**target time weighted average plasma concentration** was 52 µg/ml) [31]. Dosages for children were adjusted based on body weight to reach similar drug concentrations as those in adults. Since age and viral load are associated with risk of EVD death [32–34], the patients were grouped according to age and baseline viral loads (determined as **cycle threshold (Ct)** by qRT-PCR) for analysis. The patients aged ≥ 13 years were divided into two subgroups: Group A of Ct ≥ 20 (Ct = 20 is 5×10^7 genome copies/ml) with lower viral load (n=55) and Group A of Ct < 20 with higher viral load (n=44). Twelve young children (≤ 6 years old) were included in Group YC [28].

By the conclusion of the trial, 59 deaths had occurred within 10 days after the first dose, and 1 death at day 17. Mortality rates were 20% in Group A of Ct ≥ 20 (11 of 55, all with Ct < 25), 90.9% in Group A of Ct < 20 (40 of 44) and 75% in Group YC (9 of 12), all meeting the predefined target mortality (30% for Group A of Ct ≥ 20 , 85% for Group A of Ct < 20, and 70% for Group YC). The fatality rates in Group A were consistent with a previously observed correlation between higher viral RNA load (Ct < 20) and higher patient mortality [32, 34]. The high mortality in young children was also consistent with previous observations from two of the four treatment centers [28]. Good tolerance to favipiravir was observed during treatment, whereas continuous monitoring of viremia showed reduction in viral loads in survivors but not in non-survivors [28]. Results of available biochemical tests showed more frequent elevation of creatinine, aspartate aminotransferase and creatine phosphokinase with death in Group A Ct < 20, suggesting high levels of renal and muscular damage [28, 35, 36]. Viral clearance in the three surviving children before discharge was also observed; however, the correlations between the **secondary outcomes** of this study and treatment (Box 1) were not as apparent as those observed in adults and adolescents. Overall, this study indicated that high doses of favipiravir could be tolerated in EVD patients with Ct ≥ 20 , and furthermore, lower fatality rates observed in Group A Ct ≥ 20 suggested that favipiravir might be more beneficial during earlier stages of EVD relative to later stages. We posit that an important consideration will now be to compare patient data of Ct ≥ 20 from treatment centers with historical data, aiming to see if there is any survival advantage in such patients. This will indicate whether the enhanced survival is solely due to favipiravir treatment. If previous data is unavailable, the efficacy of favipiravir should be tested and compared in NHPs at Ct < 20 and Ct ≥ 20 .

Of note, a follow-up study reported that favipiravir plasma concentrations in 66 patients from the trial did not reach the the predefined target level 2 days after treatment initiation, the target decreasing to a median level of ~40% of the level that had actually been predicted by a pharmacokinetic model 4 days after treatment initiation [37]. In addition, no significant correlation was observed between plasma EBOV load reduction or mortality (20/66 died) and drug concentrations. This suggests that the study may have used insufficient favipiravir concentrations for the patients, and consequently, further dose studies will be needed.

Nucleic Acid-Based Therapeutics Need Optimization

Nucleic acid-based compounds represent another category of small molecule therapeutics for EVD. Two classes of nucleic acid-based systems have been reported, including antisense **phosphorodiamidate morpholino oligomers** and siRNAs. Using short oligonucleotides [15, 16], both strategies focus on targeting either viral components responsible for transcription and replication of the viral genome such as EBOV RdRp (L polymerase), or targeting antigens involved with immune suppression, such as by VP35 and VP24 [5, 6]. However, only one siRNA-based treatment has been clinically investigated.

TKM-100802 is a lyophilized nanoparticle siRNA formulation consisting of three siRNAs targeting EBOV VP24, VP35 and the L polymerase responsible for viral RNA transcription and replication [38]. In the NHP model, all four animals receiving seven doses of TKM-100802 via intravenous infusion survived challenge with a lethal dose of EBOV [39]. Following NHP studies, observations from a terminated trial in healthy adults identified an optimal dose of 0.3 mg/kg/day of TKM-100802 for safety and protective efficacy [40]. During the outbreak, it was administered to five EVD patients on compassionate grounds, but no safety and efficacy assessments could be made independently because the patients simultaneously received other treatments [41, 42].

Because the EBOV outbreak in West Africa was caused by the Makona variant of EBOV, which is distinct from the Mayinga and Kikwit variants in Central Africa, the existing product was reformulated to produce TKM-130803, with sequences specifically targeting this EBOV variant. This formulation demonstrated 100% survival (three of three) in NHPs when administered 72 h after challenge with EBOV Makona [43]. In response to the urgent need for EVD therapeutics, TKM-130803 was applied to a phase 2 trial through the Rapid Assessment of Potential Interventions and Drugs for Ebola (RAPIDE) clinical trial platform (Pan African Clinical Trials Registry PACTR201501000997429) [40]. In this non-randomized, historically controlled trial, 17 EVD patients of 18 years or older were enrolled, with 3 participants in the observational cohort and the other 14 infused intravenously with TKM-130803 at 0.3 mg/kg/day for up to 7 days. During or following infusions, no obvious cytokine release-related adverse events were observed and no termination or infusion rate change was required [40]. One patient presented exacerbated **tachypnoea** 48 h after the second dose, but the association with infusion was unclear [40]. Overall, TKM-130803 infusion was well-tolerated and survival at day 14 following drug administration was the primary outcome. Amongst the 14 drug-treated patients, 11 died, with two deaths within 48 hours after admission, and only three patients who had received 7 doses of TKM-130803 survived [40]. In the observational cohort, two of the three participants died 3 days after admission. The endpoint survival probability was 0.27 (95% confidence interval 0.06–0.58), failing to reach the pre-specified threshold of 0.55, which indicates no improvement in patient survival compared to the control cohort [40]. A possible explanation could be disease severity, as TKM-130803-treated patients all exhibited high baseline viral RNA loads (10^9 copies/ml plasma for the 11 who died) associated with fatality rates $> 90\%$ [32, 44]. In addition, 50% of the patients presented symptoms related to high fatality, including bleeding and diarrhea [45]. This suggested that there was an insufficient amount of time for the drug to take full effectiveness at the given dose and/or there was an insufficient drug

concentration in serum, even with extensive standards of care. It is possible that the potency of TKM-130803 might be improved if given at an earlier stage in EVD, but this has not been tested. Furthermore, the selected dose (0.3 mg/kg/d) may be sub-optimal for protection considering that 0.5 mg/kg/d for 7 days could only provide up to 67% protection against EBOV Makona in NHPs [40]. Moreover, the lipid formulation used in the trial was different from the one used in previous NHP studies, which may have negatively affected the efficacy of this drug in the trial. It is also not clear if siRNA uptake efficiency could have been affected due to damaged liver and/or renal functions and vascular leakage during advanced EVD [46, 47]. Of note, a study has shown that EBOV proteins VP30, VP35 and VP40 can inhibit siRNA function, possibly through interaction with the RNAi machinery and possibly blocking siRNA assembly [48]. However, whether the effect of TKM-130803 can be impeded by these viral proteins through the above mechanisms is currently unknown.

EVD Immunotherapy

IFN- β , a Proof-of-Concept Immunomodulation Trial

IFNs are important components of innate immunity against viral infection and have been used as broad-spectrum antiviral therapies. Protective effects of IFN- α , - β or - γ against EBOV have been tested in different animal models including mice [49, 50], guinea pigs [51], and NHPs [52, 53]. In one EVD patient, IFNs prepared from Sendai-virus-stimulated peripheral lymphocytes were administered intramuscularly in combination with **convalescent serum**, and this patient survived [54]. However, IFN administration in combination with other experimental therapeutics has made it difficult to assess the effect of IFNs alone. Among the few available studies on IFN monotherapy, murine IFN- γ provided up to 100% protection against a recombinant vesicular stomatitis virus expressing EBOV glycoprotein (**GP**) in IFN- α/β receptor-deficient mice [50]. In another study, six doses of human IFN- β (10.5 ug/kg) administered subcutaneously (SC) extended the survival time of NHPs challenged with EBOV or **Marburg virus**, but did not improve the survival rate [53], suggesting that IFN treatments might be beneficial, but likely not fully protective by themselves. Based on the *in vitro* observation that IFN- β could inhibit the replication of recombinant EBOV in HEK293 cells more strongly than IFN- α could [55], a **historically controlled clinical trial** tested the efficacy of IFN- β -1a in nine EVD patients in Guinea [56]. Within 2 days following qRT-PCR-mediated confirmation of EVD, IFN- β -1a (30 ug/day) was administered SC to patients daily, until patients were tested negative for EBOV, or perished [56]. Six of the patients survived in a 21-day observation window, with a survival rate of 67%, which was 2.5-fold higher than that of a control cohort treated with supportive care in the same time period at a treatment center nearby [56]. Comparison with another historical control cohort of matched age and baseline viremia showed slightly less than 2-fold higher survival in the IFN group [56], suggesting potential treatment benefit. Rapid viral clearance and improvement of certain clinical symptoms including physical strength and gastrointestinal dysfunctions were observed with IFN- β -1a treatment [56], which again, suggested a potential treatment benefit.

Inconclusive Results for Convalescent Plasma Therapy

Convalescent whole blood (CWB) or plasma (CP) is taken from patients who have recovered from EBOV infection and carry specific anti-EBOV antibodies, which has been used as prophylactic and/or therapeutic against EVD [57, 58]. Furthermore, efforts have been made to collect blood donations from convalescent EVD patients since the first EBOV outbreak in Zaire (now Democratic Republic of the Congo) in 1976, but studies on the therapeutic effects of convalescent blood or plasma on EVD are very limited. In 1977, a researcher who was accidentally infected with EBOV received human IFNs, in conjunction with two infusions of convalescent serum and eventually recovered from the infection [54]. During the 1995 EBOV outbreak in Zaire, eight EVD patients received whole blood transfusion donated by surviving patients [57]. Each patient was given one blood transfusion of 150–450 ml, 4 to 15 days following EVD onset and seven survived [57]. However, due to the combined use of other therapeutics [54], in addition to suggestions of virus attenuation late in an outbreak (which may have exaggerated any potential advantages gained from the treatment), the therapeutic benefit of convalescent blood has not been well-investigated so far and remains unclear.

During the 2013–16 outbreak, the World Health Organization prioritized the use of CWB and CP to treat EVD patients. In February 2015, a non-randomized clinical trial was launched in Guinea to evaluate the safety and efficacy of CP ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02342171) identifier: NCT02342171) [58]. Ninety-nine patients received 2 transfusions of ABO blood group-compatible CP (200–250 ml/transfusion, or 10ml/kg body weight) with a 15-min interval [58]. The source of the CP for the two blood transfusions was from separate donors [58]. Eighty-four patients who met the screening criteria were included in the final analysis of mortality and other outcomes in comparison to a historical control group of 418 patients treated with supportive care in the 5 months prior to the trial [58]. Fourteen days after treatment, 26 patients in the CP group (31%) and 158 in the control group (38%) died, and these fatality rates did not reach a pre-determined 20% difference to achieve clinical relevance, even after statistical adjustments were made based on multiple factors such as age and Ct values [58]. However, serious adverse events were not observed among the 99 patients who received CP [58]. Nevertheless, due to the unavailability of on-site methods for determining the levels of specific antibodies in CP, the quality of each transfusion (and thus efficacy) was unknown during the trial [58]. Follow-up data from this study indicated that > 90% of the CP samples contained total anti-EBOV IgG titers > 1:1000, determined by ELISA; however, only 4% contained neutralizing antibody titers of 1:160 and 75% contained a titer < 1:40 [59]. Analysis based on age and baseline Ct values revealed lower mortality in patients receiving the highest IgG doses, but also higher mortality with higher doses of neutralizing antibodies. However, neither correlation between antibody doses and mortality was significant. Thus, the effectiveness of CWB or CP-based products against EVD remains inconclusive based on the currently available data.

MAB-Based Therapeutics: A Potential for ZMapp™

Over 500 mAbs against EBOV have now been isolated from recovered patients [60–64] or developed from animal models, such as mice and NHPs [65–69]. Most antibodies are neutralizing *in vitro* and protective *in vivo* resulting in survival, but most have not yet been

tested in clinical trials. ZMapp™, a cocktail composed of three humanized mAbs targeting different sites on the surface GP of EBOV, has so far been the only one tested in clinical trials [70]. This antibody blend consists of an optimized combination of two mAb cocktails, ZMAb and MB-003, previously shown to be protective in NHPs, resulting in full survival when given at 24 h after infection, or partial survival even after the appearance of viremia [52, 71]. In a landmark study, ZMapp™ was shown to reverse advanced EVD and provide 100% protection for rhesus macaques when given up to 5 days after challenge [72]. In March 2015, a phase 1a open-label trial was launched to evaluate the safety and pharmacokinetics of ZMapp™ in healthy human adults ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02389192) identifier: NCT02389192). During the 2013–16 outbreak, ZMAb and ZMapp™ were separately given to 25 patients on a compassionate basis [73]. Twenty-two patients survived without showing serious adverse events after receiving three doses of each antibody cocktail (50 mg/kg of body weight) [73]. However, the effectiveness of the cocktails could not be accessed because the patients had also received other treatments, including CP transfusion and intensive standards of supportive care [73].

In February 2015, a randomized and controlled phase 1/2 clinical trial, the Partnership for Research on Ebola Virus in Liberia II (PREVAIL II), was initiated to evaluate the efficacy and effectiveness of ZMapp™ ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02363322) identifier: NCT02363322) [74]. The trial enrolled 72 patients (200 patients in the initial plan) from Liberia, Sierra Leone, Guinea, and the US, due to the substantial decline of EVD cases at the late stages of the outbreak [74]. The patients were randomized into either a control group receiving optimized standard of care only (oSOC, with aggressive fluid resuscitation, hemodynamic support, and other interventions available in an optimized care setting), or a treatment group receiving ZMapp™ plus oSOC (n=36 per group). The time from onset of symptoms to treatment in all patients represented 4 to 7 days. After assignment, patients received the first intravenous infusion of ZMapp™ (50 mg/kg of body weight) within 12–24 h, followed by two identical doses at every third day. The primary outcome was mortality at day 28 and data from 71 patients was included in the final analysis [74].

The fatality rates were 37% (13 of 35) in the control group and 22% (8 of 36) in the ZMapp™ group, leading to a 91.2% posterior probability of superior protection from ZMapp™, which did not reach the preset threshold of 97.5% [74]. Therefore, ZMapp™ in combination with oSOC did not show a statistically significant decrease in fatality rate over oSOC, even though mortality was 40% lower in the ZMapp™ group relative to the control group. Measurement of secondary outcomes revealed a shorter recovery period among subjects from the ZMapp™ group and the absence in most of the patients, of major safety concerns associated with antibody infusions, such as headache, myalgia, fever and blood pressure changes. These findings have suggested potential safety and therapeutic benefit.

However, for ZMapp™, an insufficient number of available EVD patients may have affected the precision of statistical analyses. In addition, the therapeutic benefit of ZMapp™ is likely underestimated, since seven of eight deaths in the ZMapp™ group occurred before the second dose of antibodies was received, suggesting that these patients may have been near, or at the terminal stage of EVD at the start of the treatment. The fatality rate (1/29) in the subgroup of patients who had finished all three doses of ZMapp™ was nearly eight times

lower than among those who survived for > 3 days since admission into the control group [74]. This suggests that additional studies are clearly needed to properly evaluate the efficacy of ZMapp™. Moreover, it is not clear whether sequence differences amongst the GPs of EBOV variants may have had any impact on the efficacy of the antibodies because of potential alterations of GP epitopes. Indeed, ZMapp™ antibodies were developed against the GP of the Mayinga EBOV variant, and comparison of the genomic sequences of the Mayinga and the Makona variants has revealed considerable genetic variations [75], including a non-synonymous mutation in the binding site for mAb 13C6, one component in the formulation of ZMapp™. Consequently, such variations might have affected the virus neutralizing effect of ZMapp™ and thus the effectiveness of this therapeutic treatment. Nevertheless, ZMapp™ might still be able to provide a survival benefit in EVD patients, but its clinical implementation warrants further investigation.

Concluding Remarks

Despite challenges of testing candidate therapeutics in the midst of EVD outbreaks, valuable experience has been gained in the design and conduct of expedited clinical trials. A common problem with the trials discussed here has been the relatively low enrollment of patients because trials may have been initiated late during an outbreak, rendering it difficult to conclude whether a specific treatment protocol presented any statistically significant benefits to patients. Non-randomized single-arm studies were conducted in most trials, where all EVD patients received experimental therapies. While ethically advantageous given that patients can receive a drug which might play a beneficial role in survival, the interpretation of perceived effects can be confounded by multiple factors, including the selection of historical controls, potential placebo-like effects, and spontaneous recovery (see Outstanding Questions and Box 1). To the best of our knowledge, the ZMapp™ trial may be the only randomized and controlled EVD clinical study that has allowed testing of this compound independently from the current standard of care protocol [76]. The flexibility in its design might enable a promising therapeutic for patients before a trial ends. While the control treatment group did not receive a drug that could be effective, patients still received standard medical care, adding a safeguard mechanism, should any unforeseen negative effects stem from the administration of therapeutics. Nevertheless, there is currently a clear lack of harmonization between trial designs for the different candidates, which makes it difficult to compare the outcomes between treatments. It will be important for future trials to have similar design in order to allow better comparisons.

Although no therapies from the trials discussed here have demonstrated statistical superiority over supportive care, it is worth noting that several EVD treatment candidates, such as favipiravir and ZMapp™, have shown a beneficial trend; this effect may reach statistical significance with further enrollment of EVD patients, or perhaps considering the elimination of patients who died early following treatment, as in the case of the favipiravir study. We posit that the evaluation of a candidate therapeutic should be adjusted based on the different stages of EVD (as determined by Ct value), since the putative compounds may have higher efficacy rates earlier in EVD, although this has not yet been directly tested (see Outstanding Questions). Alternatively, combination therapy with these single agent

treatments may be more effective, but the efficacy should be evidently tested in animal models prior to clinical evaluation.

Moreover, researchers will need to focus on how evolutionary changes in EBOV structure may affect efficacy of candidate targeting compounds. Indeed, genomic variations among EBOV variants from different outbreaks have been observed [70, 75], and genomic alterations in EBOV Makona GP and **L genes** have been shown to enhance viral transcription and replication [77], as demonstrated in luciferase reporter assays in which mutant Makona GP and L polymerase were shown to induce stronger activities in human Huh-7 cells, as well as procure a growth advantage over wild-type Makona in both Huh-7 and monkey Vero-E6 cells [77]. Furthermore, it has been proposed that EBOV genomic alterations may be associated with elevated pathogenicity and viral shedding in NHPs because the West African isolates causing the recent outbreak induced higher mortality, higher viremia level and more severe tissue injury compared to other isolates [78] (see Outstanding Questions). Consequently, surveillance of EBOV genetic variations and their impact on the efficacy of relevant therapies will be an important consideration to ensure optimized and successful therapeutic regimens for EVD patients in the future.

Of clinical relevance, recently, mAbs isolated from human survivors or immunized animals, including mouse and monkey, have shown cross-recognition of and broad protection against multiple members of *Ebolavirus* in cell lines and animal models [61, 64, 69, 79–82]. Indeed, crossreactive mAbs represent a better choice for putative therapeutics since treatments against other members of *Ebolavirus* are less developed and currently, a large range of pre-therapeutic candidates exist only for EBOV. Undoubtedly, the unpredictable nature of filovirus outbreaks highlights the importance of developing successful cross-reactive but efficacious therapeutic reagents to prevent and treat such fatal diseases associated with highly pathogenic viruses.

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Glossary

Convalescent serum/plasma

collected from convalescent patients who presumably carry specific antibodies against the pathogen causing the disease. Convalescent plasma, serum, or whole blood can be used as therapies for infectious diseases, particularly under circumstances of limited medical resources.

Ct

cycle threshold. In real-time quantitative PCR reaction, Ct refers to the cycle at which fluorescent signals from PCR amplification exceed background signals. It is a measurement

of the amount of PCR amplicon. The numerical value of Ct is inversely related to the amount of amplicons in a reaction; that is, the lower the Ct value, the higher the number of amplicons.

Escape mutant

a variant of a microorganism, such as a virus, arising through changes in genotype in response to an outside force, such as a host immune response or the effect of therapeutics.

GP

glycoprotein. For Ebola and Marburg viruses, GP is the only surface transmembrane (envelope) protein. The GP gene of Ebola virus is transcribed into two mRNAs, producing two soluble GPs (ssGP and sGP) and one full-length GP which is cleaved into structural GP1 and GP2 by cellular proteins. The Marburg virus GP gene encodes only a single GP protein. The surface GP for these two viruses play a central role in viral entry and fusion. The Ebola GP has been reported to contribute to viral pathogenesis.

Historically controlled clinical trial

a type of clinical trial in which a treated group of patients is compared to a control group treated from a past outbreak, instead of a concurrent, independent group.

L gene

the gene encoding the RNA-dependent RNA polymerase of filoviruses including Ebola and Marburg viruses. The L polymerase is approx. 220–250 kDa and is responsible for transcription and replication of the viral genome.

Marburg virus

member of the *Filoviridae* family; genus *Marburgvirus*. Similar to Ebola virus, Marburg virus is a highly infectious and fatal human pathogen. The virus was first identified in Germany in 1967 and has caused over 10 outbreaks since then. The fatality rate of Marburg virus disease can be up to 90%.

Phosphorodiamidate morpholino oligomer (PMO)

synthetic analogs of nucleic acids, approximately 18–30 subunits long. PMOs can bind to complementary RNA and block processing, and thus, are used for inhibition of gene expression.

Primary outcome

a variable that is monitored in a clinical study. Considered the most important or relevant variables to be examined in a clinical trial.

Reservoir hosts

natural hosts of an infectious pathogen; can carry the pathogen with little to no disease symptoms.

Secondary outcome

additional variable that is related to a clinical study question, but is less important than primary outcome.

Single-arm clinical study

contains only one group of participants receiving the same treatment.

siRNA

small interfering RNA: short double-stranded RNA molecule (usually 21–23 nucleotides in length) produced by RNase III-cleavage and processing of long double-stranded RNA. siRNA is assembled into a protein-RNA complex, binds to homologous sequences in mRNA and guides sequence-specific cleavage and degradation of mRNA. Some siRNAs can mediate methylation of genomic DNA and histones at loci complementary to siRNA, leading to silencing of gene expression.

Small molecule inhibitors

small chemical compounds or synthetic oligonucleotides with antiviral effects, through different mechanisms, such as interfering with the functions of viral proteins responsible for transcription and replication of a virus.

Supportive treatment

applied to manage symptoms of a disease, aiming to prevent, control or relieve symptoms or side-effects related to the treatment without targeting the underlying cause.

Time weighted average plasma concentration

the average concentration of a drug in plasma over a period of time.

Tachypnoea

or tachypnea, refers to abnormally rapid breathing. It may be a sign of more severe or advanced EBOV infection.

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Box 1**Clinician's Corner**

- Single-arm trials are advantageous in terms of ethics since all patients receive the potentially life-saving drug, but are disadvantageous scientifically since the exact impact of the drug will be unknown without a proper control group.
- Randomized, controlled trials are advantageous scientifically since a control group exists to compare the efficacy of the treatment group, but are disadvantageous ethically since not all patients receive the experimental drug.
- The primary outcome of a clinical trial for filovirus therapeutics will always be survival, since it is only possible to test efficacy on infected patients (i.e., during an outbreak).
- Two key secondary outcomes of a clinical trial for filovirus therapeutics will be the consideration of changes in viremia (RNA and live virus level) following treatment, as well as adverse effects.

TRENDS BOX

- Ebola virus disease (EVD) causes severe hemorrhagic fever in humans with high fatality rates, with no approved drugs for treatment. Several candidate therapeutics were clinically assessed during the recent 2013–2016 outbreak in West Africa.
- Two small molecule inhibitors of viral replication and transcription, a nucleotide analog (favipiravir) and short interfering RNA, did not yield a survival benefit in clinical trials, though administration of favipiravir appeared to be more beneficial for patients with lower viral loads (i.e. in the earlier stages of EVD).
- The survival benefit was inconclusive in clinical trials with immune product-based therapies, including interferon, convalescent plasma and a monoclonal antibody (mAb) cocktail. The data shows that the mAb-cocktail, ZMapp™, may have the best potential for a substantial therapeutic benefit.
- Further clinical investigations that could be rapidly initiated early during an outbreak will help conclusively evaluate the true effectiveness of available candidate therapeutics.

OUTSTANDING QUESTIONS BOX

- Have we gained sufficient knowledge from current clinical investigations into candidate therapeutics to save lives in the next outbreak?
- Is there an optimal design to better balance study strength vs. ethical concerns for testing Ebola virus therapeutics?
- Can more than one type of therapeutic be evaluated side-by-side in patients from the same trial during an outbreak?
- Given that Ebola virus infections are aggressive and the timeframe for treatment is limited, can we develop better/more sensitive diagnostic systems for the early and rapid identification of EVD?
- Can we identify markers that correlate with EVD severity so that therapeutic strategies can be personalized for more efficient treatments?
- Are we sufficiently prepared for potential threats from other filovirus species, such as Sudan, Bundibugyo and Marburg viruses?
- Do we have alternative methods for the treatment of Ebola virus **escape mutants**?
- Is cross-protection possible across all filoviruses (especially between Marburg and Ebola)?

Table 1

Clinical Trials for Candidate EVD Therapeutics

Therapeutics	Clinical trial	Treated patients	Reference treatment window	Average time from symptom onset to admission	Median/average start point of treatment	Route	Dose and course	Primary outcome	Efficacy (# deaths and mortality) and effectiveness	Adverse reactions
Avir	NCT0239054 Non-randomized, multicenter, proof-of-concept phase 2 trial [28]	111	6 d in mice [26]	1.5 d (6 y), 3–4 d (13 y)	4 d from symptom onset	Oral	6,000 mg on d 0; 2,400 mg/d, d 1–9	Mortality on-trial	60 and 54%, no difference from historical controls (58%); potential benefit to 13 y and Ct 20 (20%);	No severe adverse reactions
30803	PACTR2 0150100 0997429 Non-randomized phase 2 trial [40]	14	3 d in NHPs [43]	2 d	23 hrs post admission	Intravenous infusion	0.3 mg/kg/d for 7 d	Survival at d 14	9/12 and 75%;, no benefit	One case of worsened tachypnoea
ta	ISRCTN 1741494 6 Non-randomized, proof-of-concept phase 1/2 trial [56]	9	N/A	2–3 d	1 d upon baseline Ct determination	Subcutaneous injection	30 µg/d	Survival at d 21	3 and 33% vs 17 and 81% in control cohort; potentially beneficial	Mainly flu-like symptoms
escent plasma (CP)	Non-randomized phase 2/3 trial [58]	84	N/A	NR	48 hrs upon EVD confirmation	Transfusion	2 transfusions of 200–250 ml of CP or 10 ml of CP/kg, 15-min interval	Mortality from 3 to 16 d after diagnosis	26 and 31%, no difference from historical controls (38%); potential benefit to < 5-y (20%) or pregnancy (25%)	No severe adverse reactions
TM	NCT02342171 Randomized, controlled, multicenter	36	5 d in NHPs [72]	4–7 d	12 to 24 hrs following randomization	Intravenous infusion	3 doses of 50 mg/kg, 3-d interval	Mortality at d 28	8 (7 before the 2 nd dose) and 22% vs 37% in the control arm;	One case of hypertension

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Indications	Clinical trial	Treated patients	Reference treatment window	Average time from symptom onset to admission	Median/average start point of treatment	Route	Dose and course	Primary outcome	Efficacy (# deaths and mortality) and effectiveness	Adverse reactions
	phase 1/2 trial [74]								potentially beneficial	

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applicable reported