**EDITORIAL: EXPERIMENTAL THERAPIES FOR EBOLA VIRUS DISEASE: WHAT HAVE WE LEARNED?**

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The terrible mortality of Ebola virus disease (EVD) is most pronounced in the vulnerable groups of pregnant women and neonates. During the 2013-16 West African outbreak, hundreds of Ebola virus (EBOV)-infected pregnant women were reported, with maternal mortality estimated at over 70% and neonatal mortality nearly 100%. Thus, Dornemann *et al’s* (1) interesting case report describing an EBOV-infected neonate who not only survived but had no apparent sequelae at 8 months of age represents a first. The surviving baby, one of the last cases of EVD to be seen in Guinea, gives hope that perhaps we are finally turning the corner in finding effective treatments for this disease. Of course, one case does not constitute scientific proof of effectiveness, and it remains possible that she is simply a fortunate outlier. The report is not only instructive in itself but also raises a number of points with respect to clinical management and the assessment of investigational EVD therapeutics during the West African outbreak.

In addition to aggressive supportive care, the baby received three experimental therapies for EVD—ZMapp (a cocktail of three human-mouse chimeric anti-EBOV monoclonal antibodies), a buffy coat infusion, and GS-5734, the prodrug of a nucleoside viral RNA polymerase inhibitor. In addition, the fetus may have also been exposed *in utero* to the RNA polymerase inhibitor favipiravir, which the mother received for three days prior to delivery and her subsequent death. Thus, like most EVD patients who received care in hospitals in the United States and Europe (2) his infant received several investigational therapeutics making it very difficult to determine the impact of any particular one.

Mortality in EVD correlates directly with viral load (3;4;5), usually reflected in the field by the cycle threshold (Ct) noted on quantitative RT-PCR, the most readily available diagnostic technique. The Ct varies inversely with the viral RNA load (5). Initial review of the Ct profile of the child’s case indicates that the four ZMapp infusions were clearly insufficient to clear EBOV RNA from her blood, although it is possible that their administration controlled viral replication sufficiently to enable the child’s immune system to respond and ultimately eliminate the virus. After an initial increase in Ct value following the first ZMapp dose, it fell again, reflecting an increase in viral load.. One consideration is the emergence of ZMapp-resistant variants, as observed in EBOV-infected NHPs given another antibody cocktail MB-003(6). The eventual clearance of EBOV RNA from the blood occurred well after the last ZMapp plus buffy coat infusions and likely before use of GS-5734. Unfortunately the absence of serologic data for EBOV-specific ELISA and neutralizing antibodies during the patient’s acute course, or later during convalescence, does not allow conclusions regarding adaptive immune responses.

But just as it is impossible to attribute the child’s survival to the experimental therapies, nor should we discount their potential impact. As noted above, survival of an Ebola virus-infected neonate is extremely rare. We might be further encouraged by the 18.5% mortality seen in EVD patients who received care in hospitals in the United States and Europe, 85% of whom also received one or more experimental therapies (2), compared to case-fatalities ranging from 31-76% for patients managed in West Africa without access to these experimental therapies (7). Of course, all of the aforementioned patients received a level of aggressive supportive care, including close attention to fluid and electrolyte balance, that was not available to the vast majority of EVD patients in West Africa. But even if we conclude a causal effect for these collective interventions, an 18.5% case-fatality is still unacceptably high. More potent antivirals, likely through use of combinations, to rapidly control replication and host response modifying agents to mitigate the consequences of infection will likely be needed.

Although we would obviously like to have more conclusive efficacy data on the various experimental therapeutics for EVD, the progress made in their assessment during the West Africa should not be underestimated. Excluding a handful of patients who received convalescent whole blood in 1995 (8), the West Africa outbreak—nearly 40 years after the discovery of Ebola virus in 1976—was the first time that any experimental therapy has been administered in EBOV-infected humans, either on a compassionate use basis or in the context of a clinical trial. Based primarily on industrialized countries’ concerns of the use of Ebola virus as a bioweapon, a foundation of pre-clinical research on EVD therapeutics and vaccines has been built over the last few decades (9). Nevertheless, faced with little economic incentive and the daunting logistics of sporadic EVD outbreaks in remote locations, even the most promising products did not proceed from preclinical testing to clinical trials prior to the West-African outbreak.

The magnitude and urgency of the outbreak finally provided both a moral imperative and potential opportunity for testing experimental therapies. In August 2014, as EVD case counts in West Africa sky-rocketed, the World Health Organization (WHO) convened a meeting of medical ethicists to address the key question of whether use of experimental interventions, which had varied safety and pre-clinical efficacy profiles, was ethical given the extreme suffering in West Africa, to which the committee unanimously responded in the affirmative (10). A September 2014 WHO meeting in Geneva brought together diverse stakeholders, including representatives from the ministries of health, pharmaceutical companies, drug regulatory agencies, non-governmental organizations providing clinical care, and experts in virology, anthropology, and medical ethics to consider options for studying vaccines and therapeutics (http://www.who.int/csr/resources/publications/ebola/ebola-therapies/en/). WHO also created a Scientific and Technical Advisory Committee for Ebola Experimental Interventions to help guide the process. One of the Committee’s first objectives was to identify the most promising therapeutics among a long list of proposed candidates, including many of dubious plausibility. This process required consideration not only of the evidence for safety and efficacy, but the anticipated feasibility of conducting a clinical trial under the conditions on the ground and in the setting of limited production capacities or intermittent drug availability for some candidates. There was rigorous and sometimes contentious debate around acceptable study designs for EVD therapeutics, but eventually several novel approaches, including adaptive (11) and sequential, multi-stage trials (12) were successfully implemented (Table). These trials faced numerous challenges including relatively long delays to their being initiated, which meant that some only started late in the outbreak and were unable to include enough patients for adequate statistical power. An opportunity was also missed to enroll more patients in clinical trials in resource-rich settings. Many West African patients presented late at treatment centers by which time high levels of viral replication and associated organ damage were present, possibly reducing the therapeutic value of antiviral interventions.

There were also surprising empiric observations of apparent reduced mortality with so-called “repurposed drugs” such as artesunate-amodiaquine, routinely given to treat possible malaria co-infection, although these findings still need to be confirmed through formal clinical trials (13). Other agents like GS-5734, which is effective in controlling viral replication and salvaging NHPs when initiated up to 3 days after EBOV challenge (14), were not available in time to initiate clinical trials. However, GS-5734 was administered on a compassionate use basis to both the neonate in Guinea (1) and to a nurse with late-onset EBOV meningoencephalitis (15), who also survived.

The studies of EVD therapeutics that were successfully undertaken in West Africa, as well as the observations from compassionate use, have provided valuable results that have advanced the field. Several drug candidates progressed through early clinical trials at an unprecedented pace and the recognition that some agents are ineffective and others are promising (Table), provides a starting point for prioritization of future human studies and assessing the predictability of and improving available animal models. Yet there can be no ignoring that, despite enormous effort, we still lack a therapeutic option with proven efficacy or a clearly demonstrated clinical standard of care for EVD. Many difficult lessons were learned regarding the challenges of inconsistent reproducibility of *in vitro* experiments, inadequately predictive animal models, and the operational demands of conducting trials in Ebola treatment units during an outbreak centered in countries without pre-existing research infrastructure.

Antivirals should be assessed carefully for various indications in EBOV infection. Virus persistence and recurrent disease at immune-privileged sites like the eye and CNS has been described in several survivors, each of whom have received investigational antiviral treatments (15;16). More importantly from a public health perspective is the persistence of virus in the semen of male survivors, one of whom was implicated in reigniting an EVD cluster 470 days after his acute infection (17). Studies to test clearance of virus from semen are urgently needed, and one such trial utilizing GS-5734 have been launched in Liberia (NCT02818582). Interventions like favipiravir (18) and high doses of the recombinant vesicular stomatitis virus-vectored EBOV glycoprotein (rVSV-GP) vaccine (19) were also used for post-exposure prophylaxis in healthcare workers. However, it remains unclear whether these patients were actually exposed, so no conclusions on protective efficacy can be made; also systemic side effects occurred with the latter intervention. Lower, better tolerated doses of the rVSV-GP vaccine appeared to reduce the risk of EBOV infection in household contacts, with protection starting about six days post administration (20). In contrast, an effective antiviral could potentially provide immediate protection and/or early treatment, as established for other viral infections like HIV and influenza, and potentially could be combined with vaccine for this purpose.

Much work remains to capitalize on the lessons learned from West Africa and make the accelerated pace of clinical trials during future outbreaks the norm, including prioritizing drug candidates, completing phase 1 pharmacology and safety studies, working out trial designs and protocol details, addressing ethics committee and regulatory reviews, and setting logistical frameworks for rapid operationalization. The WHO R&D Blueprint for Action to Prevent Epidemics (<http://www.who.int/csr/research-and-development/blueprint/en/>) is one activity that is actively trying to address these parameters for a range of priority diseases before outbreaks, so that the world will have a set of tools at its disposal to rapidly evaluate experimental interventions. Discussion continues regarding the scientific and ethical merits of the various clinical trial designs used in this outbreak. For acute infections with predictably high lethality, as in EVD in pregnant women and neonates, open-label case series and sometimes individual case experiences (15) can be highly informative. We also must not forget the importance of the upstream pipeline, recognizing that the implementation of clinical trials during the West Africa 2013-16 outbreak was only possible because of years of pre-clinical research to provide viable candidates for field testing. Ongoing scrutiny of the existing therapeutic landscapes for other high consequence pathogens, support for clinical research networks to conduct studies in the inter-event period, and improved surveillance and diagnostic capacities should help to reduce response times for initiating clinical research in future outbreaks of EVD and other high threat pathogens.

**Table.** Registered clinical trials reporting results of experimental therapeutics for Ebola virus disease during the 2013-16 West African outbreak

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| --- | --- | --- | --- | --- |
| **Agent/trial name**  **(Sponsor)** | **Trial design** | **Route of administration**  **(Dose regimen)** | **Outcomes/Primary endpoint** | **Comment** |
| ZMapp (21)  PREVAIL II  (National Institute of Allergy and Infectious Diseases, USA) | Open label RCT with adaptive design, compared to optimised standard of care (SOC) alone (including favipiravir in Guinea) | Intravenous  (50mg/kg within 24 hours of enrolment followed by two more doses, every third day) | Enrolment not met (72 of 200 targeted). Mortality by day 28 post-EVD onset: 13/35 (37 %) SOC vs 8/36 (22%) SOC + ZMapp overall. Mortality among those with high virus levels (Ct < 22) at entry, 9/15 (60%) SOC vs 7/15 (47%) SOC + ZMapp. | No statistically significant survival benefit in EVD but underpowered. Infusions require 2-12 hours and may be associated sometimes with systemic reactions; these can be ameliorated by pre-treatment with antihistamines and antipyretics. Specific for Zaire EBOV and not inhibitory for other filoviruses. |
| TKM 130803 (22)  RAPIDE-TKM  (University of Oxford, UK) | Open-label, single arm with historical and concurrent controls, as part of a multi-stage approach (2) | Intravenous  (0.3 mg/kg once daily for up to 7 d) | Halted after meeting pre-specified futility endpoint (survival to day 14 of < 55%).  Survival at day 14 post-EVD onset in 3/12 (25%), after excluding 2 who died < 48 hours after enrolment. Infusions generally well-tolerated, except for one possible reaction. | No apparent survival benefit compared to historical controls, but potential confounding by enrolment of patients with high viral loads and late stage disease. Dose-limiting systemic infusion reactions (acute cytokine release syndrome) in healthy volunteers. Infused over minimum 2 hour period. EBOV Makona specific. |
| Favipiravir (5) JIKI  (Institut National de la Sante et de la Recherche Medicale, France) | Open-label, single arm with historical controls | Oral  (6g on day 1 followed by 2.4g per day on days 2-10 in divided doses)   |  | | --- | |  | | Completed.  Among 99 evaluable adults and adolescents, mortality was 20% (95% CI, 11.6%–32.4%) in those with Ct > 20 and 91% (95% CI, 78.8%–91.1%) in those with Ct < 20. RNA viral load values and mortality were not significantly different between 31 adults  starting favipiravir within <72 h of symptom onset compared to 68 who started later. No grade 3 or 4 clinical AEs. | Mortality did not significantly differ from the predefined target values of 30% for high Ct and 85% for low Ct patients. Much less active against EBOV than influenza virus in pre-clinical models. Dose regimen was approximately 2-fold higher than that tested in phase 3 trials of uncomplicated influenza but appeared to be generally well tolerated. |
| Convalescent plasma (23)  (Institute of Tropical Medicine, Belgium) | Open-label, single arm with historical controls (4) | Intravenous  (two transfusions of 200 to 250 ml of ABO-compatible convalescent plasma, with each plasma unit obtained  from a separate donor, given within 2 days of EVD diagnosis. For those < 45 kg, two  transfusions of 10 ml/kg body weight). | Completed.  Among 84 evaluable, the mortality from day 3 to day 16 after diagnosis, was 31% in the convalescent plasma group and 38% in the control group (adjusted odds ratio 0.88 [95% CI, 0.51 to1.51], adjusted for Ct values and age).  No serious AEs related to the infusions. | No overall survival benefit compared to historical controls, but levels of anti-EBOV antibodies were not determined in the plasma units. No survival or antiviral effects seen in EBOV-infected NHPs given convalescent blood with high titers of neutralizing antibodies (24) but hyperimmune globulin effective in NHPs (25). Transfusion-associated acute lung injury reported in a separate EVD patient given convalescent plasma (26). |
| Brincidofovir (27)  RAPIDE-BCV  (University of Oxford, UK) | Open-label, single arm with historical controls, as part of a multi-stage approach (5) | Oral  (200 mg as a loading dose on day 1, followed by 100 mg on days 4, 8, 11, and 15; further adjusted for patients weighing <50 kg). | Recruitment halted by manufacturer after 4 patients enrolled.  Survival at day 14 post-EVD onset in 0/4.  No serious or unexpected AEs | Variable, assay-dependent antiviral activity and selectivity for EBOV in cell culture. Antiviral action linked to brincidofovir’s lipid moiety effects on viral entry (28). No survival benefit in murine model studies at non-toxic doses. Unable to be studied in NHPs due to pharmacokinetic profile. |
| Recombinant Interferon-β1a (29) | Open-label, single arm, single center trial with historical controls | Subcutaneous  (30μg [6 x 10v6 IU] rIFN ß-1a daily for up to 10 days) | Enrolment of 9 patients. Primary outcome of blood viral load reduction based on Ct values appeared faster than controls. Mortality in 84% of 38 controls and in 33% of rIFN-β1a-treated. | Patients enrolled within 6 days of symptom onset. When analysis was conducted attempting to address differences in baseline CT values, the probability of dying for those untreated was 1.8 fold larger than for those treated. |

Abbreviations: EVD-Ebola virus disease; EBOV- Ebola virus; RCT- Randomized controlled trial; NHPs-Non-human primates; Ct, cycle-threshold value; AEs- Adverse events

Note: More detailed information on these and other investigational therapeutics is available on WHO website-

<http://www.who.int/medicines/ebola-treatment/2015_0703TablesofEbolaDrugs.pdf>

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