Ebola Virus Disease: Therapeutic and Potential Preventative Opportunities

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ABSTRACT The 2014 Ebola virus disease (EVD) epidemic in West Africa was unprecedented in its geographical distribution, scale, and toll on public health infrastructure. Standard public health measures were rapidly overwhelmed, and many projections on outbreak progression through the region were dire. At the beginning of the outbreak there were no treatments or vaccines that had been shown to be safe and effective for treating or preventing EVD, limiting health care providers to offer supportive care under extremely challenging circumstances and at great risk to themselves. Over time, however, drugs and vaccines in the development pipeline were prioritized based on all available research data and were moved forward for evaluation in clinical trials to demonstrate safety and efficacy. The armamentarium against EVD eventually included biologics such as monoclonal antibodies, convalescent plasma, and vaccines as well as small molecule therapeutics such as small interfering RNAs and nucleoside analogs. This article provides a high-level overview of the interventions and prophylactics considered for use in the outbreak and discusses the challenges faced when attempting to deploy investigational countermeasures in the midst of an evolving epidemic.

INTRODUCTION

In mid-1976 an outbreak of hemorrhagic fever was reported in southern Sudan and northern Zaire. Patients with the disease, which appeared first in southern Sudan in June 1976, presented with influenza-like symptoms including headache, fever, and myalgias and rapidly progressed to a more severe illness characterized by diarrhea, vomiting, chest pains, and hemorrhage. The disease was associated with a high mortality rate and was transmitted between close contacts of the severely ill, resulting in a substantial number of cases being linked to a local hospital (1). An outbreak of a disease with similar symptoms was noted in northern Zaire beginning in September 1976, and by 24 October there were 280 deaths out of a total of 318 cases, for a case fatality rate of 88% (2). When samples derived from patients affected by the Sudan and Zaire outbreaks were used to infect Vero cells in culture, guinea pigs, or mice, a filamentous virus similar to Marburg virus was observed (3, 4). Virus particles with a similar morphology were also identified in postmortem liver samples from patients in Zaire (4, 5). Antigenic comparisons of the new virus isolates and Marburg demonstrated that while there was cross-reactivity between the Sudan and Zaire viruses, they were distinct from Marburg virus, and the new isolates were designated “Ebola virus” (EBOV) after a river near the outbreak site in Zaire (5). Unlike Marburg virus, for which a single species has been described, at least five different species of the Ebolavirus genus exist (6). Zaire ebolavirus and Sudan ebolavirus are the species most frequently associated with human disease (7).
Before the large 2014–2015 epidemic in West Africa, outbreaks of Ebola virus disease (EVD) had occurred with some frequency, but they have been largely limited to Western and Central Africa including Uganda, Sudan, Cote-d’Ivoire, Gabon, the Republic of the Congo, and the Democratic Republic of the Congo, and most involved only a handful of cases (8). After the initial 1976 outbreak and prior to the 2014–2015 epidemic, three outbreaks stood out as exceptional due to high case numbers: the emergence of Ebola virus in Kikwit, Democratic Republic of the Congo, in 1995 (2), of Sudan virus in Uganda during 2000–2001 (10), and again of Ebola virus in the Democratic Republic of the Congo in 2007 (11).

Members of the *Ebolavirus* genus are filamentous, enveloped viruses containing linear, nonsegmented ~19-kb single-stranded RNA genomes that encode seven genes and eight proteins (12, 13); due to RNA editing, the glycoprotein (GP) gene produces separate proteins for the virion envelope GP and a secreted glycoprotein (sGP) (14). While sGP has multiple pathophysiologic and immunomodulatory roles (15), GP is critical for virus binding and fusion, and as the sole surface protein on the intact virion it is an obvious target for vaccine and monoclonal antibody development. Other structural components of the virus such as VP24, VP40, and VP35 also antagonize the innate immune response (16), activities that may be restored by blocking the interaction of the viral proteins with their cellular targets. Finally, as a single-stranded RNA virus, the Ebola virus relies on an RNA-dependent-RNA-polimerase (L) to transcribe the negative strand genome into monocistronic mRNAs for protein synthesis and a full-length antigenome as a template for replication. As might be expected, the polymerase represents a virus-specific target that can be exploited through the use of nucleoside analogs or knockdown strategies.

**MEDICAL COUNTERMEASURE DEVELOPMENT DURING THE 2014–2015 EPIDEMIC**

The successful response to any epidemic involving a disease as contagious as Ebola must focus on controlling the spread of disease through the implementation of standard public health measures, such as identifying and isolating infected persons, tracing their contacts to detect secondary infections, protecting contacts and health care workers from exposure, and ensuring the safe burial of the deceased. However, applying these public health measures on a large scale has presented complex challenges because of the limited public health infrastructure within most countries where Ebola outbreaks have occurred, and especially in West Africa when the epidemic emerged in 2014 (17). With 28,598 cases of EVD and 11,299 deaths as of 3 November 2015 (18), the unprecedented scale and speed of the Ebola epidemic in Guinea, Liberia, and Sierra Leone underscored the need for safe, effective, and rapidly deployable medical countermeasures (MCMs). These MCMs include diagnostic tests to assist in disease surveillance and case detection, vaccines to protect health care workers and help interrupt transmission, and drugs to improve the outcomes of infected patients.

Significant difficulties in successfully implementing risk communication strategies were encountered, and health care systems and Ebola treatment centers in the affected West African countries rapidly became overwhelmed (19), leading to extremely pessimistic projections on the potential course of the epidemic (20, 21). These projections added to the urgency of conducting properly designed clinical trials to evaluate a number of investigational MCMs still in early stages of development. The sooner one could establish whether an investigational MCM was safe and effective for the treatment or prevention of EVD, the sooner it could be incorporated into the response to the public health emergency (22, 23). One factor limiting the prompt evaluation of some investigational drugs was their availability, since most of the more promising antiviral candidates had been produced only in limited quantities for early development purposes. Convalescent blood and/or plasma was the exception to this rule, but the infrastructure to safely collect, store, and use this resource was not extant in West Africa until November 2014 (24), and the first clinical trials of Ebola therapeutics in West Africa were initiated in December 2014 (25).

Several therapies had been investigated *in vitro* or in animal model systems over the years that preceded the 2014 epidemic. However, at the beginning of the West African epidemic there were no treatments or vaccines that had been shown to be safe or effective for treating or preventing EVD. Clinicaltrials.gov documents only five studies of “Ebola treatments” (as compared to vaccines) prior to March 2014; these were all phase 1 safety studies (26), and of these, one was being developed for postexposure prophylaxis, not treatment. Of the four phase 1 safety studies for drugs with treatment indications, only one was completed, while the others were either withdrawn or terminated.
As the epidemic progressed, a number of different compounds, most of which had at least some activity demonstrated in animal models, were selected for evaluation in clinical trials. Product classes considered for treatment of EVD included both biologics (monoclonal antibodies and convalescent plasma) and drugs (small interfering RNAs [siRNAs], nucleoside analogs, and others).

Supportive Care
Supportive care was key to improving patient outcomes and is expected to remain a central component of the care of acutely ill patients, even in the setting of a proven specific treatment. When possible, volume replacement of patients was an important component due to intravascular depletion resulting from emesis, voluminous diarrhea, and fluid shifts within the body that deplete the intravascular space. Antiemetics and anti-diarrheals were administered when possible, as were empiric antibiotics because of possible bacteremia from transmigration of Gram-negative bacteria from the gut (27, 28). Antimalarials were also commonly administered because many patients admitted to Ebola treatment units had concomitant malaria infections. Diazepam for sedation and morphine for analgesia (29) were also commonly used. Even when sufficient medication and supplies were available, patient care suffered in the initial months because demand for health care personnel greatly exceeded what was available in affected areas (30, 31). Despite these challenges, there appeared to be an increasing level of care in West Africa over the course of the epidemic (32), with ensuing improved outcomes in the later months of the epidemic.

Supportive care delivered in developed settings, such as Europe and the United States (33, 34) also proved to be demanding. In contrast to the standard of care in West Africa, however, extensive physiological monitoring and aggressive medical interventions were available for patients admitted or evacuated to these facilities (35). A patient treated in Germany for severe EVD required blood products, ventilation, vasopressors, antibiotics and antifungals, and hemodialysis. In addition to general supportive measures, investigational interventions were provided to most patients in Europe and the United States (36), although the effects of these interventions (beneficial or harmful) could not be assessed. Not all patients needed intensive levels of support; some patients, presumably those with less severe disease, rapidly recovered after receiving only minimal support, such as intravenous fluids (37).

Therapeutics
Two American health care workers developed EVD while caring for patients in West Africa and were evacuated to the United States for medical care. In addition to a high level of supportive care, they also received the investigational compound ZMapp (35). Both patients survived, and although no conclusion of safety or efficacy could be drawn from the use of the investigational compound under these circumstances, there were widespread calls for access to early-stage investigational candidates such as ZMapp. An ethics panel advising the WHO indicated that while it was ethical to provide therapies with unproven safety and efficacy profiles, “Investigators have a moral duty to evaluate these interventions (for treatment or prevention) in the best possible clinical studies that can be conducted under the circumstances of the epidemic” so that effective therapies could be rapidly identified (38).

Clinical trials for promising treatments were put into place as rapidly as possible, but assembling the infrastructure for conducting trials took some time. In the interim, the use of investigational candidates in the United States, Europe, and West Africa continued on a case-by-case basis under what is colloquially referred to as “compassionate use”; in the United States such use is one type of expanded access that can be permitted under an investigational new drug application (39). This type of use of experimental intervention is not designed to generate conclusions about the safety or efficacy of the investigational drugs being used, given the lack of appropriate comparator groups. Unfortunately, even after clinical trials were established, access to investigational agents outside of clinical trials was advocated for by some organizations as a stop-gap measure, even though this delayed the gathering of interpretable data that would allow the most efficient identification of beneficial treatments or the rapid discontinuation of harmful therapies.

Antibodies
ZMAPP
A series of monoclonal antibodies targeting EBOV GP was developed by researchers at the U.S. Army Medical Research Institute for Infectious Diseases, and several were demonstrated to be protective in mouse models (39). Three of these antibodies (13F6, 13C6, and 6D8) were modified to deimmunize (13F6) and/or chimerize (all three antibodies) through the addition of a human Fc region. The mixture of these three antibodies (MB-003) was found to be effective in a mouse challenge model even when administered as late as 48 hours post-EBOV.
infection (40, 41). A parallel effort led by the Public Health Agency of Canada also evaluated mixtures of monoclonal antibodies directed against EBOV GP and demonstrated that a combination of three murine monoclonals (1H3, 2G4, and 4G7; ZMAb) were also effective as a postexposure intervention in mice and guinea pigs (42). MB-003 conferred a survival benefit compared to placebo when dosed after the onset of fever in rhesus macaques challenged with EBOV (43), while ZMAb protected cynomolgus macaques from an EBOV challenge when treatment was initiated 48 hours post-infection (44). The realization that a cooperative effort would benefit both groups led to a collaboration that examined whether an optimized cocktail could be formulated from the individual components of ZMAb and MB-003. As a result, it was demonstrated that a mixture of c13C6 (from MB-003) and c2G4 + c4G7 (humanized versions of two ZMAb components) provided protection even when treatment was delayed to 5 days post-infection in the rhesus macaque EBOV challenge model (45). The combination of c13C6, c2G4, and c4G7 was trademarked as “ZMapp” by MappBio and advanced as a candidate therapy for human EVD in January 2014 (http://mappbio.com/z-mapp/). ZMAb was used in at least two patients in Europe, while ZMapp was used in at least nine EVD patients (46) in the United States, Europe, and West Africa prior to the establishment of a randomized, controlled clinical trial in the United States and West Africa designed to evaluate the safety and efficacy of this investigational product in adults and children infected with EBOV (47). This trial, launched in February 2015 (48), has enrolled approximately 70 research participants at the time of this writing.

Although preliminary results with ZMapp in animal models have been promising and led to its prioritization for evaluation in clinical trials, the plant-based production system created a bottleneck that prevented timely increases in production. Initially, the ability to rapidly move the ZMAb components from a hybridoma platform to one more suitable for preparation of clinical material was considered a major advantage (49), and the use of transgenic Nicotiana benthamiana plants improved glycosylation and, thus, antibody-dependent cytotoxicity of the antibodies (41). Unfortunately, only a handful of treatment courses were available at the onset of the West African outbreak, and these were depleted by mid-August 2014 (50). While funding was rapidly made available through the Biomedical Advanced Research and Development Authority (BARDA) (46), the throughput of the facility producing the drug substance was extremely limited and was expected to produce only an additional 10 to 20 treatment courses by the end of 2014 (51).

MIL-77
Produced by a Chinese company, MabWorks, MIL-77 is a cell-derived monoclonal cocktail similar to ZMapp. MIL-77 was developed using the sequence information referenced in the ZMapp patents, raising intellectual property concerns (52). It was first administered to a United Kingdom medic who subsequently recovered from EVD, however, the efficacy could not be attributed to MIL-77 (53) in the absence of a properly designed trial, because some patients with EVD recover, especially in the setting of advanced supportive care. According to the WHO, MIL-77 is undergoing phase 1 safety trials in China (54).

Convalescent blood and plasma
Largely based on its use during prior outbreaks and the expected availability of suitable donors, in September 2014 the WHO issued a guideline recommending the use of convalescent whole blood or plasma for treatment of patients with early EVD, despite the lack of conclusive evidence of its effectiveness (55). In their guideline, the WHO noted the lack of a proven treatment for EVD and cited previous use of convalescent material to treat Ebola and other infectious diseases. Interestingly, the collection of convalescent plasma was identified as a priority for the WHO team responding to the first Ebola outbreak in 1976 (1), and units thus collected were administered to at least two patients during the 1976 outbreak (2). Early administration of convalescent serum or plasma was also documented in a 1976 case involving a researcher at Porton Down who was accidentally infected (57). The researcher, who survived, received interferon for 2 weeks plus heat-treated convalescent serum on day 3 and day 6. While there was a decrease in viremia after the first infusion of serum, the decrease cannot be attributed to the serum infusion; viremia decreases in all surviving patients at some time even in the absence of specific treatments. During the 1995 Kikwit outbreak, whole blood from Ebola disease survivors was administered to eight patients (58). There was no clear association between the volume administered or time of administration and survival, and it was noted that a controlled trial would likely be necessary to assess any treatment effect (59).

The results from animal studies evaluating the activity of convalescent plasma or other anti-Ebola hyper-immune material have been variable. An equine IgG
preparation was successful in delaying viremia, clinical signs, and death in a postexposure prophylaxis model using 1,000 plaque forming units of Ebola Zaire challenge in cynomolgus macaques but did not confer a survival benefit compared to placebo (60). In contrast, baboons challenged with a lower dose of Ebola Zaire (10 to 30 50% lethal doses) were protected if an equine hyperimmune product similar to the one described above was administered within 60 minutes of infection. No beneficial effect was observed in rhesus macaques receiving whole blood with a high titer of anti-Ebola antibodies (measured by ELISA) immediately after an Ebola Zaire challenge (56). Further complicating the overall interpretation are data indicating that neutralizing antibodies protective in one species may not be efficacious in another (61), and protection may correlate with total anti-Ebola IgG titers and not necessarily with neutralizing antibody titers (62). Antibody-dependent cellular cytotoxicity has been proposed as an important mechanism for efficacy of anti-Ebola monoclonals (41, 63) but has not been investigated in the context of the polyclonal nature of convalescent serum or plasma administered for Ebola disease.

Use of convalescent blood or plasma is not without risks, because it may result in transfusion reactions including transfusion-related acute lung injury (TRALI), hemolytic reactions, anaphylaxis, circulatory overload, and transfusion-transmitted infections. Indeed, acute respiratory distress consistent with TRALI was reported in a patient with EVD who received convalescent plasma and favipiravir on day 10 of her illness (37).

When designing a study to evaluate the efficacy of immune plasma, including a nonimmune control arm in a randomized, controlled trial is important for addressing the question of whether it is the immune component of convalescent plasma or other attributes of infusing plasma such as the hemodynamic support from an infusing fluid and protein and providing clotting factors that is responsible for the observed effects. Another factor to consider is that if immune plasma is collected from vaccine recipients rather than people who have had Ebola infection, the characteristics of the vaccine-generated hyperimmune plasma may differ from plasma collected from people who have recovered from Ebola infection. Further characterization of the similarities and differences in antibodies generated in response to natural infection and vaccination and preclinical studies may provide insights into similarities and differences and the possible clinical implications.

Three phase 2/3 uncontrolled clinical trials were initiated in West Africa with convalescent plasma, one each in Guinea, Liberia, and Sierra Leone (24). The Liberia study was discontinued due to the decline in cases in the country, but as of 19 June 2015, 101 patients had received plasma in the Guinea Ebola-Tx study (54). The investigators for the Guinea convalescent plasma trial, a historically controlled trial, reported that transfusion of convalescent plasma with unknown levels of neutralizing antibodies in 84 patients with confirmed EVD was not associated with a significant improvement in survival (64). The significant limitations of the Guinea convalescent plasma study (a historically controlled trial using plasma with unknown levels of neutralizing antibody) limit the ability to draw any definitive conclusions about the role of convalescent plasma to treat patients with Ebola. Convalescent plasma was also used under an emergency investigational new drug application for several cases of EVD in the United States prior to the establishment of the randomized, controlled clinical trial to evaluate Ebola therapies (65–67).

Small molecules

AVI-7537, AVI-7539, and AVI-6002 (Sarepta)

In 2010 the U.S. Department of Defense’s Joint Project Manager Translational Medical Technologies program awarded a contract to AVI BioPharma (now Sarepta Therapeutics) to advance their antisense-based PMOplus chemistry for therapeutics against Ebola and Marburg, building upon earlier investments in the company from the Department of Defense’s Defense Threat Reduction Agency (68). Antisense-based therapies function by binding a complementary nucleic acid strand to the mRNA encoding for a target viral protein and were first described nearly 40 years ago (69). Affinity, cellular penetration, and stability in the presence of ubiquitous nucleases presented an initial challenge, but the development of chemically modified oligonucleotides made clinical development possible (70). In 2006 phosphorodiﬂamino morpholino oligomers (PMOs) designed to target EBOV VP24, VP35, and L were demonstrated to provide pre-exposure prophylaxis against an EBOV challenge in rhesus macaques (71); a mixture of VP24 (AVI-7537)– and VP35 (AVI-7539)–directed PMOs (this combination is referred to as AVI-6002) provided protection against EBOV infection in an animal model of postexposure prophylaxis (72). AVI-6002 was advanced into a phase 1 study, where it was found to be well tolerated, albeit with a short plasma half-life (2 to 5 hours) (73). Further research indicated that this mixture of PMOs could be narrowed to a single oligomer (VP-7537) targeting EBOV VP24 and still retain potency in the rhesus challenge model (74). To date,
there are no published data on use of AVI-7537, AVI-7539, or AVI-6002 for treatment of EVD in humans, and as of 5 November 2015, further development of these compounds was in question, reportedly due to a lack of funding and intellectual property limitations (75, 76).

BCX4430 (Biocryst)

BCX4430 is a synthetic nucleoside analog of adenosine and has in vitro activity against RNA viruses in many families (including Filoviridae), as might be expected from its mechanism of action as an RNA chain terminator. This compound is active against EVD and Marburg disease in murine models of pre- and postexposure prophylaxis and protects guinea pigs from Marburg infection when dosed as late as 72 hours postinfection. Similarly, six of six cynomolgus macaques survived a challenge with Marburg when treated with 15 mg/kg BCX4430 twice a day starting at 48 hours postchallenge (77). Biocryst was awarded a 2013 National Institute of Allergy and Infectious Diseases contract to develop BCX4430 for treatment of Marburg disease and to investigate the compound’s utility for treating EVD (78). Phase 1 testing started in December 2014 and is ongoing (79); in March 2015 BARDA awarded Biocryst a contract for advanced development including clinical trials and large-scale manufacturing (80).

Favipiravir (T-705; Toyama Chemical)

Favipiravir is a pyrazincarboxamide derivative that appears to have at least some activity against a number of viruses through the inhibition of RNA-dependent RNA polymerase (81). Approved by the Japanese Ministry of Health, Labor, and Welfare in March 2014 (https://www.toyama-chemical.co.jp/eng/news/news140324e.html), the compound has been stockpiled in Japan for use against pandemic influenza (http://www.fujifilm.com/news/n150722.html). It has demonstrated activity postexposure in animal models of infection against a variety of pathogenic RNA viruses beyond influenza including West Nile virus (82), yellow fever virus (83), Lassa virus (84), and EBOV (85, 86). It has been administered prophylactically (87) to several individuals with EVD (37, 88–90). An open label, historically controlled study of favipiravir in patients with EVD (91) (the JIKI trial; NCT02329054) was sponsored by INSERM in Guinea. Based on an interim analysis of 69 patients, the authors suggest possible benefit in the subset of patients who present to care with lower EBOV viral load (92). However, limitations in the study design as well as improvements in supportive care over the course of the epidemic (32) preclude drawing any meaningful conclusions about its role in the treatment of patients with EVD.

TKM-Ebola (TKM-100802; Tekmira) and TKM-Ebola-Guinea

TKM-Ebola is a lipid-stabilized siRNA targeting EBOV VP35 and polymerase (93), while TKM-Ebola-Guinea is a version of TKM-Ebola modified to remove mismatches in the EBOV Makona variant (http://investor.arbutusbio.com/releasedetail.cfm?releaseid=907998). The use of nucleic acid–lipid particles, whose development was funded in part by the U.S. Department of Defense, had been demonstrated to be an effective postexposure therapeutic in guinea pigs and nonhuman primates, although different viral targets were examined in each study: an siRNA against L was used in the guinea pig studies, and a combination of siRNAs against VP24, VP35, and L was used in the nonhuman primate studies (94, 95).

TKM-Ebola was used on an infected French Médecins sans Frontières nurse (96) and administered to two U.S. patients under an emergency investigational new drug application (66). In December 2014 Tekmira partnered with the University of Oxford and the Wellcome Trust to perform a phase 2, single-arm clinical efficacy trial using TKM-Ebola-Guinea in Sierra Leone (http://www.who.int/medicines/news/925130). GS-5734 (Gilead)

A late breaker abstract session at the 2015 ID Week Conference described a prodrug of an adenine nucleotide analog that was effective at inhibiting growth of multiple filoviruses in cell culture (99). The presumptive target is the filovirus polymerase; a surrogate RNA polymerase was inhibited by GS-5734 with a 50% inhibitory concentration value of 1 μM. A survival benefit was demonstrated above that of placebo (50% survival in GS-5734-treated animals versus 0% survival in placebo-treated animals) when GS-5734 was administered to EBOV-infected rhesus macaques with systemic
viremia. The compound was administered to a patient with Ebola-related meningitis (100), but the contribution of GS-5734 to her recovery remains unknown.

**Vaccines**

**VSV-ZEBOV**

The recovery of recombinant vesicular stomatitis virus (VSV) from cells transfected with DNA plasmids was pioneered by Lawson et al. in 1995 and provided an ideal mechanism for rapidly growing large stocks of high-titer recombinant virus carrying foreign genes that could be used as a vaccine (101). This platform was used to generate VSVAG/ZEBOVGP, a live-virus vaccine created by substituting EBOV GP for the GP normally present in VSV (102). A single intramuscular injection of VSVAG/ZEBOVGP vaccine induced both humoral and cellular immunity and was effective at preventing EVD in cynomolgus macaques (103) challenged a month after vaccination. Protection was also observed in cynomolgus macaques challenged with the West African EBOV Makona strain as early as 3 days post-vaccination (104). The VSV-based vaccine was effective in postexposure prophylaxis animal models of infection in mice, guinea pigs, and to a lesser degree, rhesus macaques (105). This vaccine, now called VSV-EBOV, has been administered as postexposure prophylaxis to a physician who had a high-risk potential exposure to EBOV as the result of a needlestick (106). VSV-EBOV was also offered as a prophylactic measure to close contacts of the Scottish Ebola patient who experienced a recurrence of symptoms.

VSV-EBOV, originally developed by the Public Health Agency of Canada, was licensed by NewLink Genetics in 2010 and entered advanced development with support from the Department of Defense (http://investors.linkp.com/releasedetail.cfm?ReleaseID=864161) with a phase 1 study initiated in late 2014 (http://investors.linkp.com/releasedetail.cfm?ReleaseID=869082). Responding in part to concerns about scaling up production and overcoming testing delays, NewLink granted exclusive rights to VSV-EBOV to Merck (107), and BARDA provided additional funding to support manufacturing (108). VSV-EBOV elicits neutralizing antibodies and was well tolerated in healthy volunteers (109), and it is currently being evaluated in three ongoing clinical trials in West Africa: the PREVAIL, STRIVE, and Ebola ça Suffit trials. The dose of VSV-EBOV used in these trials (2 × 10⁷ plaque forming units) is consistent with that used in the rhesus and cynomolgus macaque challenge studies (1 – 5 × 10⁷ plaque forming units [103–105]). PREVAIL is a three-arm, double-blind, randomized phase 2 clinical study to compare the safety and efficacy of VSV-EBOV and ChAd3-EBOZ to placebo in the general population in Liberia (110). STRIVE also seeks to evaluate safety and efficacy, although the target population is composed of health care workers in Sierra Leone. The trial design also differs in that it is unblinded and participants are randomized to immediate vaccination or deferred vaccination (approximately 6 months later [111]). Safety and immunology results are pending for the PREVAIL and STRIVE studies, but the rapid decline in EVD cases in West Africa as the trials were ramping up in early 2015 will require efficacy assessments to be based on immunogenicity instead of disease.

The *Ebola ça Suffit* study utilizes an open-label ring vaccination strategy, where close contacts of Ebola patients are clustered into an epidemiologically defined “ring,” and each ring is randomized to either immediate vaccination with VSV-EBOV or to receive vaccination 2 weeks later, with a 1:1 ratio between the study arms (112). This design allows estimation of vaccine efficacy by comparing the hazard ratio between the two groups. As of November 2015 there are promising results from this trial (113), although there are concerns that the interim analysis may overestimate efficacy in the ring vaccination strategy. Specific concerns identified include failing to meet the pre-established statistical test of efficacy between study arms and an analytical bias due to population differences between the study arms (114).

**ChAd3-EBOZ**

Another approach for recombinant vaccines is based on the use of replication-defective adenovirus (Ad) expressing the antigen of interest. Like VSV, Ad vectors can be grown to high titers and induce a strong immune response, especially when used as the boost component in a heterologous prime-boost system; an EBOV nucleoprotein/glycoprotein (NP/GP) DNA prime/Ad-GP boost vaccine protected cynomolgus macaques against a low-dose EBOV challenge (115). However, this approach required multiple priming immunizations, which presents considerable logistical problems for effective deployment of a vaccine. An accelerated vaccination strategy was investigated, where the DNA prime was abolished and Ad vectors expressing EBOV GP or NP were used concomitantly. A single injection of the Ad-GP/Ad-NP mixture was sufficient for protection against a high-challenge dose of EBOV in the cynomolgus macaque model, and a comparison of the CD4/CD8 response pre- and postchallenge suggests that a CD8 response is important in mediating protection for this vaccine (116). Additional research indicated
that the NP component was unnecessary, and Ad-GP alone could provide robust protection in the cynomolgus macaque model (117) and also depended upon CD8 cells (118). To avoid ubiquitous pre-existing immunity to human adenovirus (which had been demonstrated to impact humoral responses to the rAd5 vaccine [119]), the vaccine backbone was changed to a chimpanzee Ad 3 (Ch3Ad). A single immunization with 10^11 particles of this construct was successful at protecting 50% of cynomolgus macaques when challenged 10 months post-vaccination. GlaxoSmithKline (GSK) and the National Institutes of Health partnered to move this vaccine candidate into clinical trials, and a phase 1 study found that while the vaccine was well tolerated, the magnitude of the immune response was less in humans than in the nonhuman primate models (120). As mentioned above, ChAd3-EBOZ is currently being evaluated in the PREVAIL trial, although results will be limited to safety and immunogenicity.

Ad26.ZEBOV (Crucell/Johnson & Johnson) and MVA-BN Filo (Bavarian Nordic)

While EBOV GP expressed in an Ad26 vector rapidly induced a T-cell response and protected against EVD in the cynomolgus macaque model (121), pre-existing immunity and duration of immunity remained a concern for the adenovirus-based vaccines. The heterologous prime-boost approach, where an adenovirus-based EBOV construct was used as the initial vaccine followed by a boost with EBOV GP expressing modified vaccinia Ankara (MVA), was promising since it provided durable immunity (122). The Crucell subsidiary of Johnson & Johnson had developed a monovalent Ebola vaccine using an Ad26 virus vector (Ad26.ZEBOV), while Bavarian Nordic had pursued a multivalent filovirus vaccine (containing the GP from Ebola, Sudan, and Marburg viruses) expressed in an MVA vector (MVA-BN Filo). An interesting collaboration to leverage the heterologous prime-boost strategy was formalized in October 2014 between these two companies (123), and multiple clinical trials are underway (http://id.bavarian-nordic.com/pipeline/filovirus.aspx).

Other vaccines

Several other vaccine candidates are also in the development pipeline. Profectus BioSciences Inc. approached the use of VSV through a different strategy than that utilized by GSK. Instead of replacing VSV G with that of EBOV, Profectus modified the vector by swapping EBOV GP for the VSV N gene, relocating the VSV N gene to a region proximal to VSV G and truncating VSV G. The resulting recombinant had a decreased growth rate in vitro and produced lower viremias in vaccinated cynomolgus macaques but was still effective at preventing EVD in this primate model with a single dose of vaccine (124). In contrast to the virally vectored vaccines advanced by Merck, GSK, Crucell, Bavarian Nordic, and Profectus, Novavax is developing a protein-based vaccine to be used with an adjuvant (Matrix-M). A two-dose regimen of the vaccine is effective at preventing EVD in cynomolgus macaques (http://novavax.com/download/files/presentations/Novavax_EBOV_GP_Vaccine_2015_07_21_FINAL.pdf), and Novavax initiated a phase 1 study for a recombinant GP protein vaccine in February 2015 (125).

CONCLUSIONS

The development and evaluation of investigational therapies for an emerging infectious disease such as Ebola requires a number of elements to be in place, ranging from the ability to produce or manufacture sufficient quantities of good-quality investigational agents so that clinical trials to evaluate the investigational agents can be conducted, infrastructure to provide care for patients and to support the conduct of clinical trials, engagement of the affected communities in the response effort, information on the disease and its major manifestations, and properly designed clinical trials that have the capacity to draw scientifically valid conclusions and protect patient safety. The epidemic of EVD in West Africa revealed serious weaknesses in international preparedness and response efforts to emerging threats.

The manufacturing or production of sufficient supplies of investigational product to support the conduct of clinical trials was a major challenge in the response to the Ebola epidemic. The reasons for these delays differ for the different types of investigational products that are being developed for Ebola. For example, delays in planning, organizing, and equipping health care providers with the means to collect convalescent serum and blood (identified as one of the highest therapeutic priorities by the WHO [126]) was one factor that impeded the evaluation of these potential therapies. When developing therapies for an emerging infectious disease such as Ebola, it is important to consider the product characteristics, the ability to scale up production, and the time required to deliver adequate supplies in response to an outbreak. For products that are not currently stockpiled, availability and scalability of the production process must be taken into account when prioritizing MCMs or developing contingency plans for their use. These risks can be mitigated in part by
ensuring that appropriate mechanisms exist for enlisting additional manufacturing facilities and/or filling lines when necessary.

The characteristics of an investigational product and the setting in which it will be used impact the utility of the product in the response effort. The drugs and vaccines developed by the United States were initially envisioned for use in the context of prophylaxis of military personnel or for a bioterrorist event resulting in cases of EVD, and their use assumed a high degree of coordination between local, state, and federal partners within the United States. Monoclonal antibodies such as ZMapp must be slowly administered over several hours to decrease the risk of infusion-related adverse events. For outbreaks where the ability of health care providers to provide and monitor the administration of compounds such as ZMapp is compromised, other product classes that can be delivered orally or as an intramuscular injection may be preferable. Likewise, stability can complicate deployment efforts in areas with sporadic electricity and refrigeration. For example, the VSV-EBOV vaccine requires ultra-low (−70°C) storage for long-term stability; experience with polio and smallpox vaccination campaigns demonstrated the importance of a thermally stable vaccine. In the absence of such a stable vaccine, the ability to maintain a cold chain is essential and must be part of contingency planning. This planning should also consider how the vaccines and drugs can be evaluated for safety and efficacy during the outbreak to rapidly identify the safest and most efficacious MCMs.

For therapies against an emerging infectious disease such as Ebola, it is important to consider biological diversity and that the infectious agent may acquire mutations that could alter targets for countermeasures. It would be ideal to have multiple countermeasures with different mechanisms of action in order to have therapies that will remain active in the setting of mutations of the infectious agent or species differences that may impact the activity of some countermeasures. For example, monovalent vaccines developed based on EBOV strains such as Mayinga or Kikwit protect against the Mayinga strain, but had the outbreak been triggered by a separate Ebola virus species (Sudan ebolavirus or Bundibugyo ebolavirus), cross-neutralization would be unlikely and the vaccines would require modification. Similarly, MCMs that are highly specific to specific strains and species of virus are vulnerable to the emergence of sequence variants. TKM-Ebola was modified to TKM-Ebola-Guinea to more closely match the sequence of the Makona strain of EBOV. Therefore, stockpiles of monovalent MCMs may be less valuable for response than those composed of multivalent vaccines and antivirals with broad activity.

Demonstrating the safety and efficacy of a new drug or vaccine can be a challenging endeavor under normal circumstances. These challenges were amplified by the rapidly evolving Ebola epidemic (a communicable agent causing severe illness with significant mortality), the unprecedented stress on the West African health care system and society in general, and the need to establish infrastructure to support clinical trials to identify beneficial therapies. Properly designed clinical trials are an essential component of the response effort. The findings from such trials can help patients by identifying whether an investigational therapy benefits patients. Conducting clinical trials that are not properly designed can delay the identification of effective therapies, lead to uninterpretable or misleading conclusions, and impede the ability to adequately monitor patient safety. For example, the lack of well-designed clinical trials and information about the characteristics of the convalescent plasma tested have impeded the ability to evaluate the role of convalescent plasma in treating patients with EVD. Fortunately, there were some successful and partially successful efforts during the epidemic. Some well-controlled trials of vaccines and a therapeutic were implemented during the epidemic. While these trials were implemented very quickly compared to the usual time to launch a clinical trial, it is apparent that we need to continue to build on this effort so that the evaluation of countermeasures can be implemented even more rapidly in the response to any future outbreak.

The importance of the advance preparation of protocols to allow the evaluation of safety and efficacy of MCMs in an outbreak setting cannot be overstated. Randomized clinical trials offer advantages in terms of rapidly providing interpretable, robust data on the safety and efficacy of an MCM (127). Careful consideration must be given to inclusion and exclusion criteria, and if need be, flexibility must be introduced into the protocol to allow for adaptation; for example, the PREVAIL I protocol was modified in November 2015 to include an open-label cluster vaccination component in response to new clusters of EVD in Liberia. The use of a common protocol that allows the flexibility for evaluating multiple unproven MCMs is one potential solution to quickly identify the most effective therapy or prophylactic while weeding out those that actually cause harm, and it can help communicate a standard level of supportive care that is expected for patients (22). If there are debates about how to ethically conduct certain trials,
these conversations should take place before an outbreak instead of during the outbreak. Community engagement also has a role to play in trial design. When the purpose for randomized, controlled trials—to evaluate the safety as well as efficacy of untested products—was articulated to local populations by individuals trusted by those in the community, there was acceptance as evidenced by enrollment and high visit compliance in the Ebola ça Suffit and PREVAIL vaccine trials (110, 113). In addition, the above-mentioned cluster vaccination component of the PREVAIL I protocol was implemented by the Liberian partners, building on the training they had received for the initial trial.

Sadly, for the first time in history, a sufficient number of EVD cases existed to allow for the collection of data on the natural history of disease in humans. Applied research to address knowledge gaps benefitted from a great deal of collaborative work accomplished through partnerships between nongovernmental organizations, industry, academia, and government agencies. While the data thus generated will be analyzed and subjected to scrutiny and debate for years to come, we do not have the same luxury of time in which to apply the lessons of the world responded to this unprecedented outbreak. Considering severe acute respiratory syndrome–associated coronavirus, pandemic influenza, and now EVD, we must do more in terms of preparedness and contingency planning for every stage of the MCM product development cycle. Instead of asking “What do we have?” during an outbreak, the question should be reframed to “Which contingency plan is appropriate?” for any given outbreak situation.

ACKNOWLEDGMENTS

This book chapter reflects the views of the authors and should not be construed to represent the FDA’s views or policies.

REFERENCES

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