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Ebola Virus: Pathogenesis and Countermeasure Development

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Abstract

Since its discovery in 1976, Ebola virus (EBOV) has caused numerous outbreaks of fatal hemorrhagic disease in Africa. The biggest outbreak on record is the 2013–2016 epidemic in west Africa with almost 30,000 cases and over 11,000 fatalities, devastatingly affecting Guinea, Liberia, and Sierra Leone. The epidemic highlighted the need for licensed drugs or vaccines to quickly combat the disease. While at the beginning of the epidemic no licensed countermeasures were available, several experimental drugs with preclinical efficacy were accelerated into human clinical trials and used to treat patients with Ebola virus disease (EVD) toward the end of the epidemic. In the same manner, vaccines with preclinical efficacy were administered primarily to known contacts of EVD patients on clinical trial protocols using a ring-vaccination strategy. In this review, we describe the pathogenesis of EBOV and summarize the current status of EBOV vaccine development and treatment of EVD.

INTRODUCTION

Ebola virus (EBOV) is the most prominent member of the *Filoviridae*, a family of enveloped viruses with a single-stranded, negative-sense RNA genome of approximately 19 kb (1). The genome encodes seven distinct genes from which at least nine proteins are expressed: nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), soluble GP (sGP), small soluble GP (ssGP), transcription activator (VP30), minor matrix protein (VP24), and RNA-dependent RNA polymerase (L) (**Figure 1**). The GP gene transcription results in three different mRNAs leading to the expression of full-length GP (cleaved by furin into GP1 and GP2), sGP (delta peptide cleaved off by furin), and ssGP. The *Filoviridae* is divided into three genera: *Ebolavirus*, *Marburgvirus*, and *Cuevavirus* (1). Five distinct species are known in the genus *Ebolavirus*: *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï forest ebolavirus*, *Bundibugyo ebolavirus*, and *Reston ebolavirus*, represented by EBOV, Sudan virus (SUDV), Taï forest virus, Bundibugyo virus (BDBV), and Reston virus, respectively (2). In the last decade, EBOV, SUDV, and BDBV have caused Ebola virus disease (EVD) outbreaks with increased frequency and case fatality rates from 30% to 90% in central and west Africa (3). Recently, a new ebolavirus, Bombali virus (BOMV) in the proposed new species *Bombali ebolavirus*, was discovered in bats in Sierra Leone (4) and has also been found in bats in Kenya (5). Similarly, Mengla virus (MLAV) was identified in fruit bats in China (6). It is not known whether these viruses cause disease in humans or nonhuman primates (NHPs), as only sequence information is available, and no virus has been isolated thus far. In contrast, the genus *Marburgvirus* contains only one known species, *Marburg marburgvirus*, consisting of Marburg virus (MARV) and Ravn virus. Likewise, the genus *Cuevavirus* has one species with one known virus named Lloviu virus. Similar to BOMV and MLAV, only sequence information is available, and no virus has been isolated from infected bat samples in Europe (7, 8).

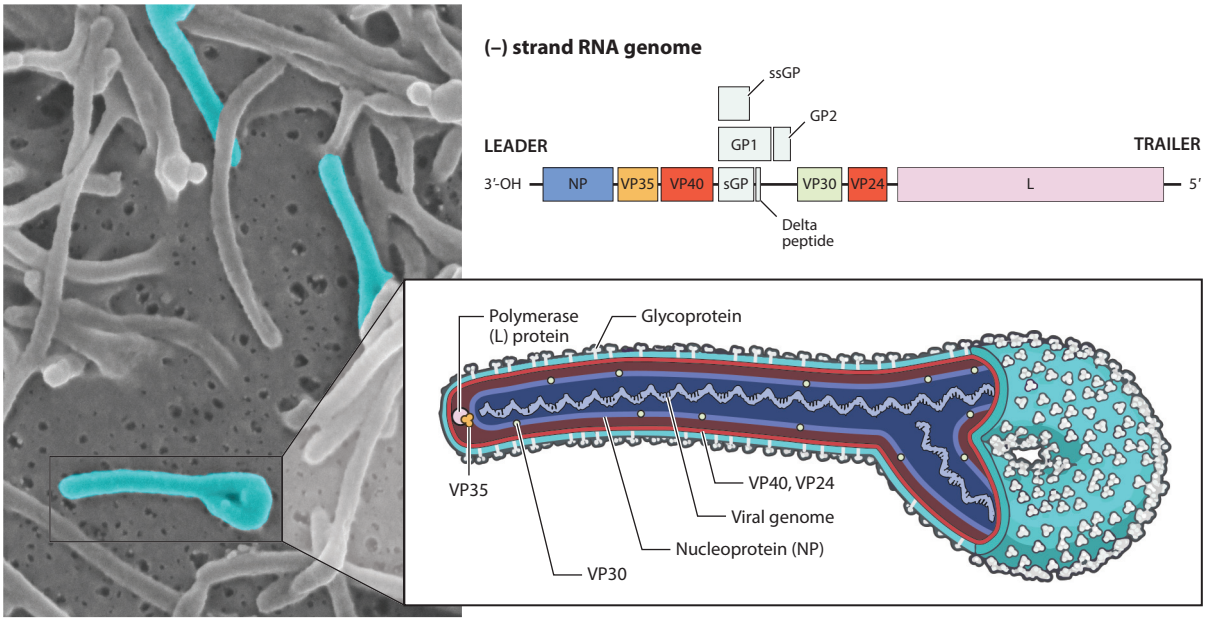

Figure 1

Electron micrograph and schematic of the EBOV particle and genome. EBOV particles (*blue*) on the surface of an infected cell are shown. Abbreviations: EBOV, Ebola virus; GP, glycoprotein; sGP, soluble glycoprotein; ssGP, small soluble glycoprotein; VP, virion protein.

EBOV causes severe hemorrhagic fever in humans and NHPs, with human case fatality rates of up to 90% (1). The first outbreak of EVD occurred in the Republic of Zaire [now the Democratic Republic of the Congo (DRC)] and southern Sudan in 1976. From these independent outbreaks, two distinct viruses, EBOV and SUDV, were identified (9, 10). In 2008 from human isolates collected during an outbreak in Uganda, another human-pathogenic ebolavirus, BDBV, was identified (11). EBOV, SUDV, and BDBV have been responsible for numerous small, self-limiting outbreaks in central Africa, with maximum case numbers in the hundreds (12). However, the 2013–2016 EBOV epidemic in west Africa, which originated in Guinea and spread to Liberia and Sierra Leone, resulted in 11,323 fatalities among at least 28,646 cases (13, 14). The epidemic accelerated efforts to develop antiviral strategies, and some experimental therapeutic and vaccine candidates were evaluated in clinical trials. During this epidemic, several experimental drugs also were used for the treatment of EVD patients (13). Furthermore, the two preclinically most-promising vaccine candidates, vesicular stomatitis virus (VSV)-EBOV and chimpanzee adenovirus (chAd)-EBOV, were deployed toward the end of the epidemic in phase 3 clinical trials with the hope of interrupting human transmission chains using a ring-vaccination approach (15, 16). But even after these efforts, no effective treatment for EVD is commercially available. However, China and Russia were the first to license an EBOV vaccine in 2018 (17).

This review describes the pathogenesis of EBOV infection in humans and animal models and covers the current state of development of therapeutics and vaccines.

EBOLA VIRUS PATHOGENESIS

Ebola Virus Disease in Humans

Infection of human index cases starting EBOV outbreaks is mainly linked to spillover events occurring during hunting wildlife, exposure to animal carcasses found in the forest, or contact with the putative virus reservoir, bats. These initial infections result in subsequent human-to-human transmissions, which account for 99% of all human EVD cases (18). Symptoms of EVD in humans normally occur after an incubation period of 2–21 days (19–21). There are typically three phases of illness; it starts with a few days of nonspecific fever, headache, and myalgia, followed by a gastrointestinal phase in which diarrhea and vomiting, abdominal discomfort, and dehydration are prominent (**Figure 2**). In the advanced and final stage of disease, liver and kidney function decline, often causing severe metabolic compromise, convulsion, shock, and death due to mucosal bleeding, bloody diarrhea, and multi-organ failure within 16 days after the first symptoms appear (19, 21, 22). EBOV initially replicates in antigen-presenting cells, such as macrophages and dendritic cells (DCs) (23). However, the virus can infect many cell types, including macrophages, monocytes, DCs, Kupffer cells, fibroblasts, hepatocytes, and cells of adrenal gland tissue as well as endothelial and epithelial cells, which may all contribute to the increase in viremia over time (23, 24). Cell dysfunction and death caused by EBOV have been hypothesized to play an important role in the signs and symptoms of EVD, such as failure of the immune system to respond adequately to the infection or decreased production of clotting factors (21). Furthermore, several immunological mechanisms are involved in the pathogenesis of EBOV infection, including inhibition of type I interferon (IFN) responses, deregulation of the cytokine/chemokine network, and the functional impairment of DCs and natural killer (NK) cells.

The type I IFN family is a multigene cytokine family that encodes 13 partially homologous IFN-α subtypes, a single IFN-β, and several poorly defined single gene products in humans (25). Type I IFNs have diverse effects on innate and adaptive immune cells for host defense against virus infections (25). Early IFN-α production during infection correlates with survival in both

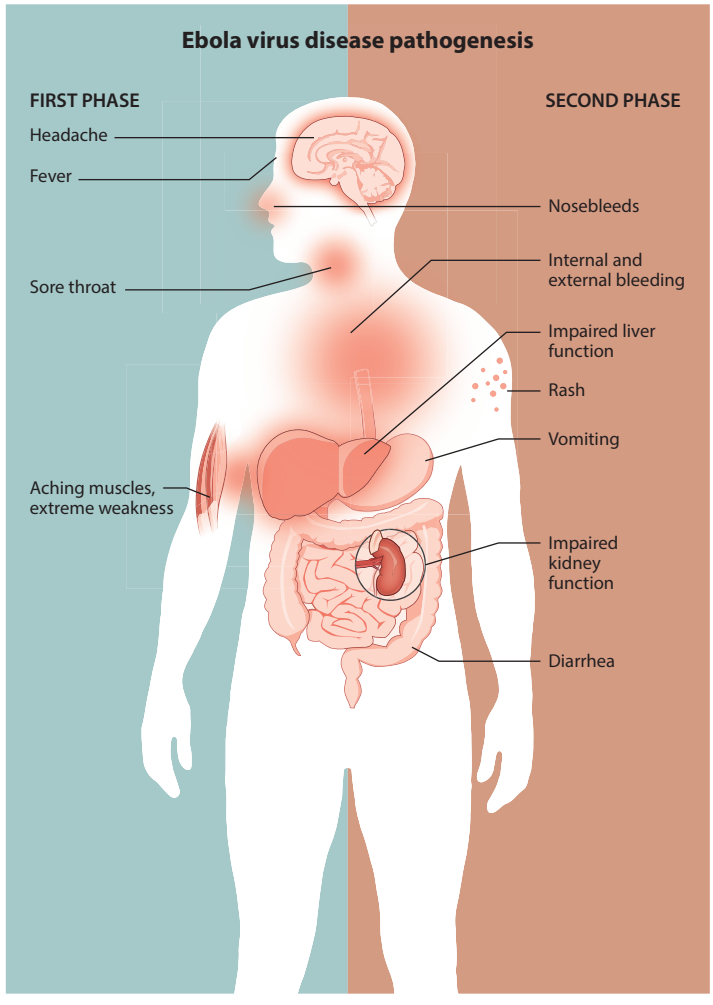

Figure 2

Ebola virus disease pathogenesis. The parts and functions of the human body affected during the first and second phases of the disease are highlighted.

mice and humans infected with ebolaviruses (26, 27). However, EBOV counteracts the protective effects of IFN-α and IFN-β (28, 29). EBOV infection of peripheral blood mononuclear cells fails to induce type I IFNs and inhibits IFN-α production induced by double-stranded RNA (dsRNA) (30). The EBOV VP35 suppresses IFN-β production via multiple inhibitory effects that include disruption of the RIG-1 pathway and inhibition of activation of IFN-inducible dsRNA and Dicer-dependent protein kinase R (31–33). Furthermore, EBOV VP24 disrupts both type I (IFN-α and IFN-β) and type II IFN signaling by inhibiting the dimerization of phosphorylated STAT proteins and, therefore, blocks transcription of antiviral genes (34). Thus, EBOV has evolved to counteract the host immune response, resulting in an advantageous environment for replication and progeny virus production.

While early cytokine responses are correlated with survival from EVD, at a later stage of disease, EBOV infection induces hypersecretion of numerous cytokines (interleukins IL-1β, IL-1RA,

IL-6, IL-8 IL-10, IL-15, and IL-16 and TNF-α), chemokines, and growth factors (MIP-1α, MIP-1β, MCP-1, M-CSF, MIF, IP-10, GRO-α, and eotaxin) (30, 35). A 5- to 1,000-fold increase of these proinflammatory mediators compared to their normal range can be measured during EBOV infection, creating a so-called cytokine storm (36). The dysregulated inflammatory response is associated with organ failure, sepsis syndrome, and ultimately death. However, if an infected person can mount a controlled inflammatory response early after infection that is able to control virus replication and spread, adaptive immune responses will be initiated and can lead to survival of the infected individual (36).

Several inflammatory mediators are induced within the first hour following EBOV exposure even prior to viral gene expression, suggesting a direct role for the GP in inducing an initial inflammatory response (35). Additionally, during EBOV infection, a significant amount of EBOV GP is shed from infected cells in a soluble form due to cleavage by the cellular metalloprotease TACE (TNFα-converting enzyme) (37). This shed GP is released from virus-infected cells and activates noninfected DCs and macrophages, contributing to the massive release of pro- and anti-inflammatory cytokines affecting organ function including vascular permeability (38). As the disease progresses, abnormal production of selected lymphokines and cytokines induces several pathological disorders including apoptosis of bystander lymphocytes, tissue damage, and loss of vascular integrity, which likely contribute to virus-induced sepsis and shock. Furthermore, the abnormal systemic inflammation of the small blood vessels may progress to an active process of fibrin deposition, platelet aggregation, coagulopathy, and liposomal release from stagnant leukocytes inside the vascular system (39, 40).

DCs, which are the initial target cells of EBOV (23), are also an important mediator of innate and adaptive immunity. However, EBOV proteins VP24 and VP35 promote aberrant expression of cytokines and chemokines and, therefore, impair the differentiation of DCs (41). By blocking DC maturation, EBOV inhibits the activation of lymphocytes including NK cells and, therefore, eliminates the immune cell subsets that could mount an antiviral response. Furthermore, EBOV-infected DCs fail to produce cytokines themselves, including type I IFNs, and are unable to mature correctly and, therefore, are unable to induce proper B cell, NK cell, and T cell responses that could control EBOV replication (42, 43). In fact, during EBOV infection, the populations of NK cells and other lymphocytes decrease in humans (30). In fatal human cases of filovirus infection, the downstream effects of antigen-presenting cell dysfunction are measurable and correlate with a marked lack of adaptive immunity, likely contributing to the fatal outcome (44).

Ebola Virus Disease in Animal Models

The continued development of EBOV countermeasures depends heavily on animal models that recapitulate the disease observed in humans. A variety of animal models of EBOV infection have been used for basic research, characterization of pathogenesis, and development of antivirals and vaccines. Antiviral and vaccine candidates are often first evaluated in one or more small-animal models (screening models) such as mice, guinea pigs, and hamsters (45–48) because they permit preclinical evaluation and have predictive value for testing in NHPs and, therefore, help to conserve precious NHP resources. Ferrets have more recently been used as models for EBOV disease, but limited availability of reagents has hampered the thorough characterization of disease in these animals thus far (49, 50). Since NHPs offer the most accurate recapitulation of human EVD, these animals are considered the gold-standard filovirus animal model and are used for all confirmatory testing (51).

Mice are the most commonly used animal model in the EBOV field for many reasons: They are easy to handle in the Animal Biosafety Level 4 laboratory, there are a lot of tools for analyses of host

responses, and transgenic and knockout strains are readily available for research. However, wild-type EBOV (WT-EBOV) isolates do not cause clinical signs of disease in adult immunocompetent mice (52, 53). Therefore, to study disease, investigators have used immunodeficient mouse strains mainly lacking a proper type I IFN system, such as STAT1$^{-/-}$ or interferon α/β-receptor$^{-/-}$ mice or severe combined immunodeficient (SCID) mice. These immunodeficient mice are very susceptible to infection with WT-EBOV, as a low dose is sufficient to cause predominantly lethal disease within 1 week after infection (52). Interestingly, the virus replicates mainly in the liver and spleen, the key tissues of EBOV infection in NHPs and humans (52). Furthermore, these studies demonstrate that the type I IFN response plays a key role in the susceptibility to EBOV infection of mice (52, 54, 55). However, SCID mice remain healthy for approximately 14 days following WT-EBOV infection but then develop gradual, progressive weight loss along with reduced activity and eventually succumb on days 20–25 postinfection (52). While these mice can be used to rapidly test the efficacy of candidate countermeasures against a new outbreak isolate, the immunocompromised status of these animals has disadvantages (e.g., the mice do not mount normal immune responses to vaccines).

To establish an EBOV mouse model showing disease in immunocompetent mice, researchers sequentially passaged WT-EBOV nine times in progressively older suckling mice (56). The resulting mouse-adapted EBOV (MA-EBOV) is lethal for commonly used adult immunocompetent mouse strains [e.g., Balb/c, CD-1 (ICR), and C57BL/6 mice]. Intraperitoneal (IP) inoculation with 1 or 100 plaque-forming units results in uniform lethality and approximates 30 or 3,000 times the median lethal dose (LD_{50}), respectively (56). Moreover, despite not causing disease, WT-EBOV can replicate in the liver and spleen of adult immunocompetent mice. However, replication was ~1,000-fold lower compared to the MA-EBOV under the same conditions. This observation suggests that both viruses share the same initial target cells (56). In addition, MA-EBOV-infected mice exhibit widespread lymphocyte apoptosis, which is a hallmark of EVD in humans (57–59). Interestingly, MA-EBOV infection does not cause disease in immunocompetent mice when the virus is administered by the intramuscular (IM) or subcutaneous (SC) route; only IP inoculation of the virus results in uniform disease and lethality (56). Compared to WT-EBOV, MA-EBOV acquired eight mutations in the coding and noncoding regions of the virus genome, with amino acid changes occurring in the GP, VP24, VP35, NP, and L genes (60). Determinants of virulence in mice include coding mutations in NP and VP24 (60). Overall, the mouse model of EBOV infection demonstrates high viremia and viral loads in the spleen, liver, and other tissues (52, 61). In addition, lymphopenia, kidney dysfunction, and liver damage result in high serum concentrations of aspartate aminotransferase and alanine aminotransferase, observations that correlate with disease progression in humans (52, 59, 61, 62). However, commonly used mouse models exhibit little to no other hallmarks of EBOV infection in humans such as coagulopathy, tissue fibrin deposition, disseminated intravascular coagulation (DIC), or the characteristic maculopapular to petechial rash. The model is primarily used to determine the efficacy of countermeasures prior to further evaluation in NHPs. However, some countermeasures with demonstrated efficacy in mice do not protect NHPs from lethal disease, highlighting the limited predictive value of the mouse model for efficacy studies using NHPs (48).

Guinea pigs also are widely used for EBOV research. Inoculation of guinea pigs with WT-EBOV results in a mild febrile illness with partial lethal outcome (63). Since WT-EBOV infection of guinea pigs causes only a mild illness with partial lethality, WT-EBOV also was serially passaged through liver and spleen samples to select guinea pig–adapted EBOV (GPA-EBOV) (47). The adaptation enhanced the virulence in guinea pigs, reflecting the pathomorphological changes of the infection until it resulted in uniform lethality (64). Like MA-EBOV, GPA-EBOV acquired mutations in VP24 sufficient to cause lethal disease in these animals (65). Infection of guinea pigs

with GPA-EBOV results in fever, thrombocytopenia, and increased fibrin deposition until day 5. However, fibrin depositions, DIC, and a maculopapular rash are not regularly observed in this model (47, 66–68). Furthermore, despite the severe lymphopenia during GPA-EBOV infection, lymphocyte bystander apoptosis, which is an important feature of infection in mice and NHPs, generally does not occur in guinea pigs. However, the guinea pig model has a better predictive value regarding countermeasure efficacy studies in NHPs compared to mice (69–72).

Similar to mice and guinea pigs, adult Syrian golden hamsters also are resistant to WT-EBOV infection. However, a hamster model for EBOV was developed by inoculating hamsters IP with MA-EBOV (73). MA-EBOV replication was systemic, with high virus titers detected in the blood, spleen, liver, kidney, heart, lung, and brain. MA-EBOV-infected hamsters developed signs of severe disease, such as ruffled fur and decreased activity, beginning on day 3 postinoculation, and all animals died within five days postinoculation. Importantly, this is the only small-animal model of EVD that exhibits severe coagulopathy. Coagulopathy is preceded by an initial abnormal increase in fibrinogen concentration, representing the acute-phase response to infection. Due to the presence of rash and induction of cytokines/chemokines, the Syrian hamster recapitulates hallmark signs of EVD better than guinea pigs. However, this model is still not widely used in the EBOV research community despite its higher predictive value for countermeasure efficacy testing in NHPs due to limitations in available reagents.

The domestic ferret was established as an EBOV animal model even though these animals are more difficult to handle in the maximum containment laboratory compared to rodents. While there are only limited reagents available to study pathogenesis and immune responses in ferrets, these animals have the advantage that they develop disease after inoculation [intransal (IN) and IM] with WT-EBOV (49, 50). Important manifestations of EVD in NHPs occur in ferrets, including petechial rash, reticulated pallor of the liver, splenomegaly, hemorrhaging at the pyloric/duodenal junction and in lymph nodes, thrombocytopenia, and elevated liver enzyme levels (49, 50). Following EBOV infection, these animals develop terminal disease, resulting in similar transcriptomic profiles when compared to NHPs and humans (74), providing further validation of this model.

NHPs such as marmosets, cynomolgus macaques, rhesus macaques, African green monkeys, and hamadryas baboons are preferred for studies of EBOV infection, since these animals are susceptible to infection with WT-EBOV and display disease attributes and pathology similar to those seen in humans with EVD (75). However, mainly macaques are used due to their relative ease of acquisition, handling, and sampling. As in humans, EBOV spreads from the initial infection site via monocytes/macrophages and DCs to regional lymph nodes and to the liver and spleen through the blood stream (23). Furthermore, EBOV antigen is detected in Kupffer cells and cells lining the sinusoids in the liver during the early stages of infection, followed by antigen detection mainly in hepatocytes during later stages (76). EBOV activates DCs by upregulating expression of tumor necrosis factor–related apoptosis-inducing ligand, which is expressed on DCs and mediates their cytotoxic activity (23). Inoculation of NHPs with EBOV can result in body temperatures above 40°C and pyrexia, which usually persist throughout the course of the disease and which end in a decrease in body temperature at the terminal stage of disease followed by death within 5–8 days postinoculation (77, 78). Furthermore, increases in liver enzyme levels and the proinflammatory cytokines IL-6, TNF-α, and IFN-γ have been detected (23). Additionally, NHPs develop thrombocytopenia, neutrophilia, and DIC, which is characterized by prolonged coagulation times, decreased protein C levels, increased fibrin degradation products (D-dimers), and increased tissue factor expression (23, 79, 80). Petechial skin rashes appear on the head, limbs, chest, and abdomen 4–7 days postinoculation in macaques and baboons but not in African green monkeys (81). In African green monkeys, fibrin thrombosis is generalized in all visceral organs, while in baboons,

hemorrhages were prominent in visceral organs, most notably in the liver and spleen (80, 82). While both cynomolgus and rhesus macaques are equally susceptible to EBOV infection, the disease course is slightly delayed in rhesus macaques, resulting in an extended treatment window of ~1–2 days. Therefore, cynomolgus macaques are most often used for vaccine studies, whereas rhesus macaques are more frequently used for evaluation of therapeutics (83).

VACCINE DEVELOPMENT

Vaccine Platforms and Preclinical Efficacy

Vaccination is considered a primary strategy for infectious disease control in humans. Therefore, it is no surprise that the first attempts to develop an EBOV vaccine started shortly after the discovery of the virus in the late 1970s (84, 85). The first vaccine to be developed against EBOV was inactivated virus, which protects guinea pigs from lethal disease (86). Since then, the preclinical development of a variety of different vaccines against EBOV commenced and included DNA, virus-like particles (VLPs), recombinant viral vectors, and recombinant proteins (87). The efficacy of each vaccine candidate has been evaluated in rodents or NHPs, with several approaches conferring 100% protection against lethal EVD (87). There are eight vaccine candidates currently in human clinical trials. These vaccines all target the EBOV GP but differ in the predominant immune response they elicit, the antigen delivery system, and the side-effect profile (**Table 1**). In the following text, we elaborate about the most successful vaccine candidates.

The first DNA vaccine for EBOV was developed using plasmid encoding sGP and GP, which elicited humoral and T cell responses (88). DNA vaccines have several advantages relative to live-attenuated vaccines. They are safe to use and easy to produce, the DNA itself induces immune-stimulatory responses, and the host-cell protein synthesis allows for endogenous presentation of the desired antigen. The first successful immunization strategy using a DNA vaccine against EBOV was described in 1998 and showed that 100% of mice were protected from lethal EBOV challenge when given four doses of a DNA vaccine encoding either EBOV GP or EBOV NP (89). DNA vaccination with optimized antigen expression resulted in 83% protective efficacy in NHPs (90). However, a combination of a DNA prime together with an adenovirus boost, both encoding EBOV GP, showed 100% protective efficacy in NHPs (91). An updated EBOV GP-based DNA vaccination approach resulted in uniform protection when three doses were administered to NHPs (92). Preliminary data from a human trial using this DNA vaccination strategy against EBOV reported desirable safety profiles, and vaccinees developed adequate levels of immune responses after the two to three doses, underlining the need for at least one booster to achieve protective immunity (93).

Ebola VLPs, which are morphologically similar to infectious EBOV particles, have also been explored as vaccine candidates. They are produced by coexpression of EBOV GP and VP40 in transfected cells; the EBOV NP also can be present in these preparations but is not required for protective efficacy. GP and VP40 undergo self-assembly, and the resultant VLPs bud from transfected cells. VLP vaccination results in enhanced stimulation of NK cells, which play a crucial role in innate immune protection against lethal EBOV infection (94). In addition, VLP vaccination triggers host responses through Toll-like receptor and type I IFN signaling, leading to initiation of early innate protective immune responses (95). In rodent models, VLPs consisting of VP40 and GP were 100% protective against lethal EBOV infection (96–98). Furthermore, vaccination with these VLPs in combination with the RIBI adjuvant induced EBOV GP-specific antibodies and strong T cell responses, and all vaccinated NHPs survived lethal EBOV challenge without clinical signs (99). Another more elaborate subunit vaccine candidate is the replication-deficient EBOV

Table 1 Vaccine platforms in clinical trials

Vaccine platform	Vaccine class	Immunogen(s)	Pro(s)	Con(s)	Clinical trial(s)
Protein	Subunit	GP	Safety	Production can be difficult and expensive	Phase 1
DNA	DNA	GP, NP	Safety, flexible platform, low cost	Effective administration requires electroporation technology; requires boost vaccination for immunogenicity	Phase 1
Ad26	Replication-deficient virus expressing EBOV protein	GP	Safety, low preexisting immunity to vector	Requires boost vaccination	Ad26/cAd3; phase 1 Ad26/MVA; phases 1, 2, 3
Ad5	Replication-deficient virus expressing EBOV protein	GP	Safety, induced the strong cellular and humoral immunity	Preexisting immunity to vector, requires boost vaccination	Ad5; phases 1, 2 Ad5/VSV; phases 1, 2, 4
chAd3	Replication-deficient virus expressing EBOV protein	GP	Safety, low preexisting immunity to vector	Requires boost vaccination	chAd3; phases 1, 2 chAd3/MVA; phase 1
MVA	Replication-deficient virus expressing filovirus protein	GP, NP	Safety	Immunogenicity: requires heterotypic pairing for vaccine efficacy	Ad26/MVA; phases 1, 2, 3 chAd3/MVA; phase 1
VSV	Replication-competent virus expressing EBOV protein	GP	Single vaccination is highly immunogenic and fast acting	Some safety concerns	Phases 1, 2, 3
HPIV3	Replication-competent virus expressing EBOV protein	GP	Intranasal administration may elicit more robust mucosal immunity	Preexisting immunity to vector	Phase 1

Abbreviations: Ad, adenovirus; chAd, chimpanzee adenovirus; EBOV, Ebola virus; GP, glycoprotein; HPIV3, Human parainfluenza virus 3; MVA, modified vaccinia Ankara; NP, nucleoprotein; VSV, vesicular stomatitis virus.

lacking the VP30 gene (EBOVΔVP30). This virus can be propagated only in VP30-expressing cell lines, is genetically stable, and very closely resembles EBOV (100). Inoculation of mice with EBOVΔVP30 resulted in robust EBOV GP-specific antibody and EBOV NP-specific T cell responses, and all animals survived lethal challenge with MA-EBOV. A follow-up study showed that guinea pigs immunized twice with EBOVΔVP30 were protected from lethal challenge with GPA-EBOV (101). Most importantly, immunization with one or two doses of EBOVΔVP30 protected NHPs against lethal infection with EBOV (102), suggesting that EBOVΔVP30 is an effective EBOV vaccine. Indeed, this vaccine platform is currently moving toward a human phase 1 clinical trial (103).

Viruses that are generally not associated with human disease have been engineered as antigen-delivery vehicles and have become increasingly popular because of their capacity to induce strong cell-mediated immune responses. The antigens encoded by these recombinant viruses are

expressed and processed intracellularly from infected cells. The most advanced EBOV vaccines in preclinical development are viral vectors encoding EBOV GP. These vectors can be either replication competent or replication deficient. A replicon system based on Venezuelan equine encephalitis virus (VEEV) is effective against EBOV challenge. VEEV replicons expressing EBOV GP protect guinea pigs and mice from lethal EVD (104). In addition, a single IM vaccination with VEEV replicon particles expressing SUDV GP combined with VEEV replicon particles expressing EBOV GP resulted in complete protection against lethal challenge with either SUDV or EBOV in NHPs (105). However, we are not aware of any plans to test this vaccine in human clinical trials.

Adenoviruses are nonenveloped, double-stranded DNA viruses that are capable of infecting many mammalian species. Human adenovirus serotype 1, serotype 2, and serotype 5 (Ad5) are common and cause mild upper respiratory tract infections. Replication-deficient human Ad5 has been used as a vaccine platform for EBOV and other pathogens (106). Ad5 is easy to manipulate, replicates to high titers, and induces strong cellular and humoral immunity against the encoded antigen. Replication-deficient recombinant Ad5 used alone as a high-dose vaccine or as a boost vaccine following a DNA prime showed 100% protective efficacy in NHPs against lethal EBOV challenge (91, 107). However, because Ad5 is a common human pathogen, preexisting immunity diminishes the efficacy of Ad5 vaccines and limits their human use. To circumvent this problem, an alternative strategy was developed using other Ad serotypes that rarely circulate in humans (e.g., Ad26 and Ad35). In addition, a chAd vector with a low human seroprevalence has been explored. Studies using NHPs demonstrated 75% efficacy of a single dose of Ad26-EBOV vaccination against lethal EBOV challenge and 100% efficacy when this vector was used with an Ad35-EBOV boost (108). A single dose of recombinant chAd3 expressing EBOV GP protected 100% of NHPs against lethal EBOV challenge 5 weeks after vaccination. However, the protection decreased to 50% when animals were challenged 10 months postvaccination (109). To improve the durability of protection, a chAd3-EBOV prime was boosted with a modified vaccinia Ankara (MVA) vector encoding the EBOV GP (MVA-BN Filo) 8 weeks later. This combination protected all animals when challenged 10 months after the last vaccination (109). The MVA-EBOV vector expressing GP is not uniformly protective on its own (109), but it is currently in human clinical trials in combination with different Ad vectors (110). The MVA platform has been optimized by expression of EBOV VP40 as a second antigen in addition to GP. This vector is uniformly protective in macaques after a single-dose vaccination (111). Several human clinical trials with Ad and MVA are currently ongoing.

Promising vaccine candidates have been developed based on replication-competent viral vectors. A VSV-based EBOV vaccine is among the leading EBOV vaccine candidates. VSV is a member of the *Rhabdoviridae* family and causes disease in livestock and other animals. It is highly restricted by the human interferon response and generally does not cause any or only very mild disease in humans (112). Recombinant VSV-based vaccines induce strong innate and humoral immune responses, are easily propagated in cell culture, and undergo little if any genetic recombination or reassortment (113). The recombinant VSV-EBOV vaccine (also known as rVSV-ZEBOV) was engineered by replacing the VSV-G coding sequence in the wild-type VSV genome with a sequence encoding EBOV GP. This chimeric virus displays no neuropathogenicity compared with wild-type VSV (114) while still replicating efficiently in cells (115). The protective efficacy of the VSV-EBOV vaccine against lethal EBOV challenge has been extensively analyzed in rodents (15). In NHPs, VSV-EBOV conferred protection against IM and aerosol EBOV challenge 4 weeks after vaccination, and protection was afforded with various routes of vaccination (IN, IM, or oral) (116–118). Moreover, VSV-EBOV conferred 100% protection in NHPs when administered within 1 week of lethal EBOV challenge (119). These data are intriguing, as they suggest that the vaccine

can be used as an emergency vaccine, which was shown to be successful during the end of the epidemic in Guinea (detailed in the section title Vaccines in Human Clinical Trials).

Recombinant rabies virus (RABV), which belongs to the same family as VSV, also has been developed as an EBOV vaccine. A single immunization with a replication-competent recombinant RABV expressing EBOV GP conferred partial protection in mice against lethal EBOV challenge. Complete protection of NHPs was achieved by two immunizations with this vaccine candidate (120, 121). However, a replication-deficient or an inactivated version of this vaccine candidate provided 50% protection (121). The inactivated RABV vaccine vector was improved using a codon-optimized antigen, resulting in higher-titer neutralizing antibody levels and 100% protection against lethal EBOV challenge when applied with adjuvant (122). The vaccine is moving toward human clinical trials.

In addition to rhabdovirus-based viral vectors, recombinant paramyxovirus-based vectors have been developed as EBOV vaccine candidates. Human parainfluenza virus 3 (HPIV-3) belongs to the genus *Respirovirus* in the *Paramyxoviridae*, a family of single-stranded, negative-sense RNA viruses that are being investigated as vaccine vectors. Multiple GPs can be inserted into the HPIV-3 genome (123). Single-dose immunization of recombinant HPIV-3 expressing EBOV GP alone or GP and NP together protected guinea pigs against lethal EBOV challenge (124). Additional studies showed that a single immunization with a construct expressing only the EBOV GP was moderately immunogenic and protected 88% of NHPs against EBOV challenge. A two-dose immunization protocol using the same vaccine was highly immunogenic, and all NHPs survived lethal challenge (125). Mucosal (IN/intratracheal) administration of the HPIV-3-EBOV GP vaccine also elicited robust immune responses and provided protection from disease after IM EBOV challenge in NHPs (126).

Vaccines in Human Clinical Trials

The development of EBOV vaccines started in 1977, shortly after the discovery of EBOV, and continued with renewed force after 2001 when more funding became available to engineer countermeasures against pathogens with bioterrorism potential. Despite these efforts, most of the vaccine candidates remained in the preclinical stage, as there was limited interest from pharmaceutical companies and other institutions to license such products with only a few thousand human EVD cases since 1976. Only four EBOV phase 1 vaccine trials had been conducted evaluating either the DNA or the rAd5-based vaccines in the United States, and all of them were conducted by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) (127–130). This situation changed with the 2013–2016 EBOV epidemic in west Africa when clinical trials for EBOV vaccines were accelerated, resulting in more than 60 clinical trials registered on **https://ClinicalTrials.gov** or the Pan African Clinical Trials Registry (131). Most trials analyzed safety, immunogenicity, and efficacy of chAd3, Ad5, DNA, HPIV3, subunit, chAd3 combined with MVA-BN Filo, Ad26-EBOV combined with MVA-BN Filo, VSV-EBOV, or VSV-EBOV combined with Ad5-EBOV vaccine candidates (**Figure 3**). Ad26-EBOV combined with MVA-BN Filo, VSV-EBOV, and VSV-EBOV combined with Ad5-EBOV were assessed in phase 1–3 clinical trials (**Table 1**).

Seven phase 1 clinical trials using a prime-boost regimen with a combination of the Ad26-EBOV and MVA-BN Filo vaccines are either ongoing or completed in different countries, including the United States, United Kingdom, and several African countries (**https://ClinicalTrials.gov**; NCT02325050, NCT02313077, NCT02376400, NCT02891980, NCT02376426, NCT02376426, NCT02860650). In one phase 1 trial with healthy volunteers, immunization with Ad26-EBOV or MVA-BN Filo (NCT02313077) did not result in any serious vaccine-related

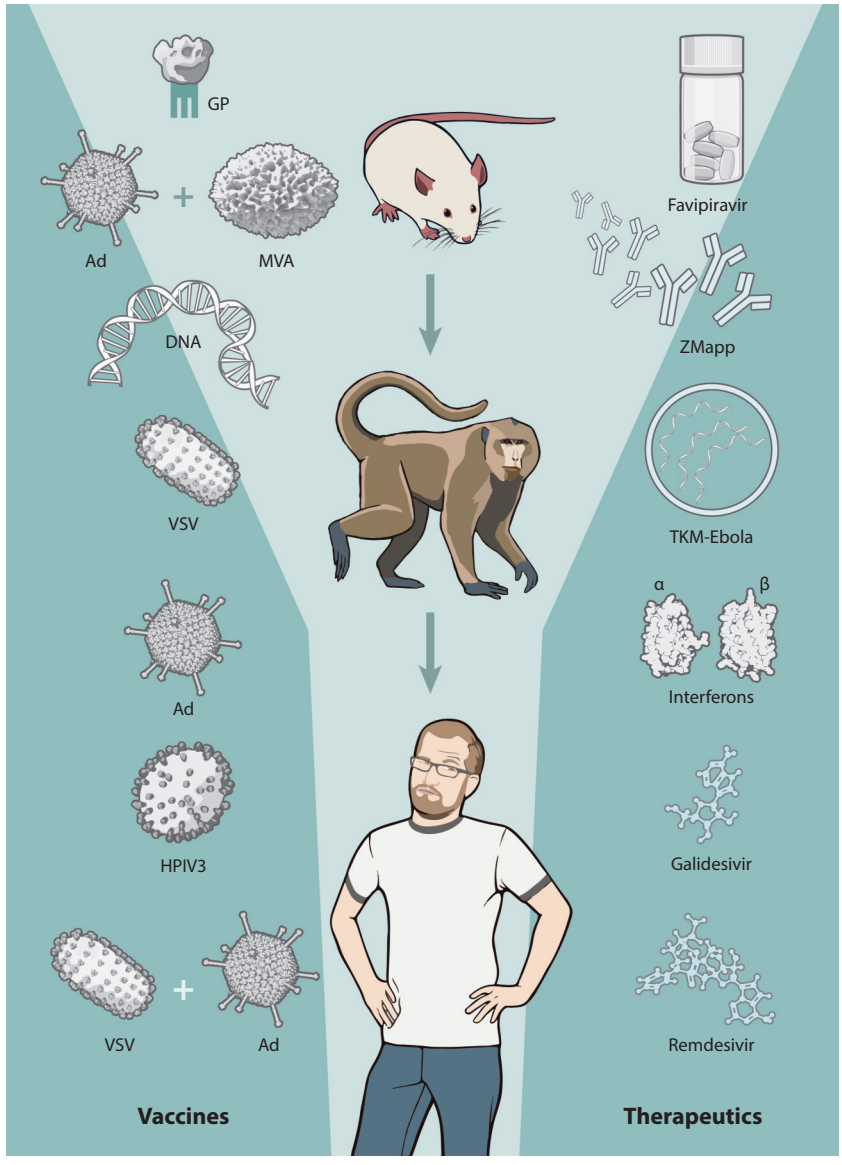

Figure 3

Vaccines and therapeutics in human clinical trials against Ebola virus disease. Vaccine and therapeutic approaches with preclinical efficacy undergoing human clinical trials are shown. Abbreviations: Ad, adenovirus; GP, glycoprotein; HPIV3, human parainfluenza virus 3; MVA, modified vaccinia Ankara; VSV, vesicular stomatitis virus.

adverse events. The observed safety profiles were acceptable, and durable cellular and humoral immune responses were measured up to 8 months after vaccination (132). In addition, stable immune responses to the vaccine candidate were observed after 1 year. All vaccines maintained EBOV GP-specific antibody responses, and 60% to 83% of the trial participants showed vaccine-induced T cell responses at that time (133). Furthermore, four phase 2 random, observer-blind, placebo-controlled, parallel-group, multicenter trials using the same prime-boost regimen in

healthy adults, children, elderly participants, and human immunodeficiency virus (HIV)-positive adults also are ongoing or completed in the United States, France, and several African countries (NCT02564523, NCT02598388, NCT02416453, NCT02876328). Further assessment of the safety and immunogenicity including durability of the Ad26-EBOV vaccination regimes is either ongoing or was completed in the United States (NCT02661464, NCT02543567, NCT02543268), and a large phase 2/3 trial is in progress in Sierra Leone (NCT02509494, Pan African Clinical Trials Registry No. PACTR201506001147964). Results of these trials have not been reported as of this writing.

GamEvac-Combi, the combination vaccine of VSV-EBOV and Ad5-EBOV, was developed in Russia. One open-label, dose-escalation phase 1/2 clinical trial in 84 healthy adults of both sexes between 18 and 55 years was conducted in Russia (134). This vaccine candidate induced EBOV GP-specific antibodies in 95% of the volunteers, and neutralizing antibodies were detected in 93.1%. Furthermore, CD4+ and CD8+ T cells were detected in 82.8% and 58.6% of participants, respectively (134). This trial was registered with **https://grls.rosminzdrav.ru** (No. 495) and **http://zakupki.gov.ru** (No. 0373100043215000055). A more recent trial tested the immunogenicity of a lyophilized vaccine (NCT03333538). The GamEvac-Combi vaccine approach is now licensed in Russia. However, there are limited preclinical data for this vaccine approach, and only one phase 1/2 clinical data set has been published. The vaccine is being tested in an ongoing phase 4 clinical trial in Guinea and Russia (NCT03072030).

Since the EBOV epidemic, numerous human clinical trials with VSV-EBOV are ongoing or have been completed in North America, Europe, and Africa (15, 110), and still others are planned. Ten phase 1 clinical trials of VSV-EBOV were conducted to evaluate the safety and immunogenicity of the vaccine as well as to identify doses and regimes that may be evaluated further in phase 2/3 clinical trials (NCT02269423, NCT02280408, NCT02283099, NCT02374385, NCT02287480, NCT02296983, NCT02314923, PACTR201411000919191, NCT02718469, NCT02933931) (135–142). Following this work, five phase 2 trials using the VSV-EBOV vaccine candidate in healthy adults, HIV-positive adults, and adults at risk for EBOV infection are also ongoing or were completed in the United States, Canada, Liberia, Sierra Leone, and Guinea (NCT02344407, NCT02378753, NCT02788227, NCT02876328, NCT03031912) (143). In 2015, three phase 2/3 or 3 clinical trials were conducted. The first phase 3 trial was an open-label, cluster-randomized, ring-vaccination phase 3 trial in Guinea to assess the efficacy of the VSV-EBOV vaccine administered IM for the prevention of EVD during the epidemic with the goal to interrupt human transmission chains (PACTR201503001057193) (144, 145). In the first clinical trial, 80 serious adverse events were identified, of which two were judged to be related to vaccination (one febrile reaction and one anaphylaxis) and one possibly unrelated (influenza-like illness). All three individuals recovered without sequelae. The second trial was an open-label, individually randomized, controlled phase 2/3 trial conducted in Sierra Leone (NCT02378753, PACTR201502001037220) (146). The third study was a randomized, double-blind, multicenter phase 3 clinical trial in the United States, Spain, and Canada (NCT02503202) (147). These clinical trials support the use of VSV-EBOV as an emergency vaccine in individuals at risk for EVD, including health-care workers, first responders, direct contacts of confirmed EVD cases, and contacts of such contacts. An interventional, single-arm, open-label, nonrandomized, phase 3b study has been conducted in Uganda and the DRC to accumulate additional data about the safety and effectiveness of one dose of VSV-EBOV against EVD (NCT03161366). In the DRC during the May–July 2018 EBOV outbreak, contacts of confirmed EVD patients received the vaccine in a ring-vaccination approach. During the ongoing largest outbreak in North Kivu of the DRC, VSV-EBOV is the only vaccine being used and has been administered to over 93,000 individuals, again with the goal to break human-to-human transmission chains (148).

THERAPEUTIC INTERVENTION STRATEGIES

Since there are no effective, commercially available treatments for EVD, care for those with this disease relies on basic supportive treatment such as fluid and electrolyte replacement, oral antibiotics and antimalaria treatment, and control of pain and other symptoms. However, even early on after the discovery of EBOV, convalescent plasma was used to treat infected patients with disputable efficacy. The plasma is obtained from survivors and contains polyclonal antibodies targeting EBOV. During the EBOV outbreak in Kikwit in 1995, eight persons were treated with convalescent plasma. However, from the study it is not clear whether the treatment contributed to the survival of seven of the treated individuals, as other factors might have contributed (149). During the west African EBOV epidemic, this potential treatment was revisited. Convalescent plasma was evaluated in human clinical trials for its safety and efficacy for the treatment of EVD patients, but a beneficial effect on survival was not observed (150, 151). There are many unknowns regarding this treatment, including the timing of when to collect the plasma, the amount of IgG reactive with EBOV, and the titer of neutralizing antibodies in the plasma. In addition, there is the risk of serum-sickness reactions, which reduces the feasibility of this approach.

In 2014, the World Health Organization (WHO) issued a document on the categorization and prioritization of drugs for consideration for testing or use in patients infected with EBOV, which has been frequently updated since then (152). There are five different drug categories described in the document, most importantly category A drugs that are being evaluated in clinical trials in west Africa (**Table 2**). This category includes the four most promising treatment approaches: ZMapp, favipiravir, TKM-100802 (TKM-Ebola), and IFNs (**Figure 2**).

Monoclonal antibody (MAb) therapy is now commonly used to treat EVD after an individual becomes infected, as the antibody targets the virus and inhibits replication at early stages of virus entry into host cells. Several neutralizing MAbs protect rodents from EBOV infection, although early studies found that the single neutralizing MAb KZ52 failed to protect NHPs from lethal disease (153). Single MAb therapy with MAb114 demonstrated 100% preclinical efficacy in NHPs (154, 155) and is currently being tested in a clinical trial protocol in the DRC (NCT03719586). Beginning in 2012, several studies demonstrated partial to complete efficacy of antibody treatment in NHPs using MAb cocktails or purified polyclonal IgG (156–158). In particular, a combination of three humanized EBOV GP-specific MAbs called ZMapp demonstrated a high level of protection in NHPs when given as late as 3–5 days after lethal challenge (72). During the 2013–2016 epidemic, ZMapp was given to EVD patients on a compassionate-use basis, and most of the

Table 2 Therapeutics in clinical trials

Therapeutic	Characteristic	Target viral protein	Route	Clinical trial
ZMapp	Cocktail of three humanized monoclonal antibodies	EBOV GP	Intravenous infusion	Phases 1, 2, 3
Favipiravir (T705)	Nucleic acid analog	RNA-dependent RNA polymerase	Oral	Phase 2
TKM-100802 (TKM-Ebola)	Small interfering RNAs	EBOV polymerase L, VP24, and VP35	Intravenous infusion	Phase 1
BCX4430 (galidesivir)	Nucleic acid analog	Viral RNA polymerase	Intramuscular injection	Phase 1
GS-5734 (remdesivir)	Nucleic acid analog	Viral RNA polymerase	Intravenous infusion	Phases 2, 3
AVI-6002	Combination of antisense phosphorodiamidate morpholino oligomers	EBOV VP24 and VP35	Intravenous infusion	Phase 1

Abbreviations: EBOV, Ebola virus; GP, glycoprotein.

patients survived without showing serious adverse events following three doses of the antibody combination (159). In March 2015, a phase 1a open-label trial was launched to evaluate the safety and pharmacokinetics of ZMapp in healthy human adults (NCT02389192). This product is moving toward licensure and is currently being used in the DRC on a multidrug clinical trial protocol (NCT03719586). While ZMapp is an attractive therapeutic option against EVD, it is expensive to produce, which is problematic in Africa where health-care funds are limited. It is possible that new strategies for MAb production and delivery will expand the utility of ZMapp and similar preparations.

Another therapeutic approach with efficacy against EVD is the purine nucleic acid analog, favipiravir (T-705), which is a broad-spectrum antiviral agent that is licensed in Japan and currently in phase 3 clinical trials in the United States for the treatment of influenza (160). While favipiravir protects mice from lethal EBOV infection (161), no survival benefit was observed in NHPs in two studies using once or twice daily oral treatment during EBOV infection. However, the treated NHPs showed extended time to death and reduced viral RNA loads, suggesting an antiviral effect of favipiravir against EBOV (162). During the epidemic, favipiravir was the only drug meeting three important criteria required for human trials: an antiviral effect in animal models, a good safety profile in humans, and a large supply of the drug readily available for human use. By meeting these criteria, favipiravir was quickly moved into a noncomparative proof-of-concept trial, in which all patients received favipiravir along with standardized care (163). In 2014, favipiravir was given to 39 patients with EVD admitted to the Sierra Leone–China Friendship Hospital. Patients with confirmed EVD were treated with either WHO-recommended supportive therapy (control group) or WHO-recommended supportive therapy and favipiravir. There was a benefit of favipiravir treatment in long-term survival, but the results were not statistically significant (164). Several other nucleic acid analogs, including BCX4430 and remdesivir (GS-5734), also have been evaluated in preclinical studies for the treatment of EVD (165, 166) and were assessed in human clinical trials during the epidemic (NCT02319772). Remdesivir currently is being used in a human trial protocol in the DRC (NCT03719586). These inhibitors are less expensive per dose. However, they can lead to adverse effects after administration, which may outweigh the antiviral benefits.

Small interfering RNAs (siRNAs) efficiently inhibit EBOV replication *in vitro* (69). Therefore, Tekmira Pharmaceuticals Corp. (now Arbutus Biopharma) developed TKM-Ebola, which is a combination of siRNAs targeting expression of three EBOV proteins, the polymerase L, VP24, and VP35. TKM-Ebola demonstrated efficacy against EBOV in NHPs (70, 167) and was administered to two adult patients under a compassionate-use agreement in combination with extensive supportive care and convalescent plasma. The two patients survived despite severe disease-related clinical and biological alterations (168). However, a phase 1 clinical trial demonstrated severe dose-related side effects, including dizziness, chest tightness, and tachycardia. These side effects occurred even when the dose was decreased, and clinical development of this drug was stopped (152). Another nucleic acid–based drug, AVI-6002, is a combination of antisense phosphorodiamidate morpholino oligomers targeting expression of VP24 and VP35. In 2011, a phase 1 clinical trial showed that AVI-6002 was well tolerated by healthy human volunteers (169). There is no information about further clinical development of this treatment approach.

IFN-α and -β are type I IFNs, a family of cytokines with antiviral, antiproliferative, and other immunoregulatory properties (25). EBOV infection is associated with several alterations in the host immune response, including downregulation of type I IFN and massive lymphocyte apoptosis (28, 29). Therefore, it was postulated that IFN administration would help control the infection and attenuate the associated unregulated inflammatory host responses. Two studies were conducted to evaluate the efficacy of IFN monotherapy against EBOV infection in NHPs. The results showed that IFN monotherapy as a postexposure regimen had no overall effect on outcome but

appeared to prolong time to death (170, 171). However, when combined with an antibody mixture, Ad-vectored IFN-α was efficacious in NHPs (172). In fact, nine patients with EVD were treated with IFN-β-1a in Guinea (173). Within 2 days following confirmation of EVD, IFN-β-1a was administered SC to patients daily for 17 days. The survival rate was increased from 19% (receiving supportive care only) to 67% (receiving supportive care plus IFN-β-1a), demonstrating a beneficial effect of the drug (173). However, the limited data gathered in studies of NHPs and humans do not offer much support for IFNs as monotherapy against EVD.

CONCLUSION AND PERSPECTIVE

Over 40 years after the first appearance of EBOV in Yambuku, DRC, the country is facing its largest EBOV outbreak with over 1,000 cases and >50% case fatality rate (148). In the past, outbreaks of EVD in the DRC were managed well, resulting in rapid containment of EBOV, as demonstrated during the May 2018 outbreak in Bikoro, DRC (174). However, the ongoing outbreak is located in an area of civil unrest, hampering national and international humanitarian efforts to contain and end the outbreak. While we learned much about EVD and potential vaccines and treatments during the epidemic in west Africa, there are still no licensed products available in Europe and the Americas. Only Russia and China have licensed an EBOV vaccine (20). Therefore, what can be done to improve the situation in the DRC beyond political stability? There have been over 93,000 people vaccinated with VSV-EBOV (148) with the intention to interrupt human transmission chains. In addition, three therapeutics are being tested in a clinical trial protocol (NCT03719586) to treat EVD patients. Will the use of countermeasures on a compassionate-use basis continue in future outbreaks, or can we hope to have the VSV-EBOV vaccine licensed soon together with ZMapp and other therapeutics? While Africa may not be the most appealing market for pharmaceutical companies, EBOV can appear in other countries, which was observed during the west African epidemic, highlighting the need for globally available vaccines and therapeutics. Governments in endemic countries should consider stockpiling products to control outbreaks before they spread and become a huge humanitarian and economic burden. Such an approach also should appeal to pharmaceutical companies and might speed up licensure of vaccines and therapeutics currently administered under compassionate-use agreements.

EBOV is not the only reemerging pathogen with epidemic potential; Lassa virus (LASV), Middle East respiratory syndrome virus, and Crimean Congo hemorrhagic fever virus (CCHFV) exhibit expanding endemic areas and frequent introductions into the human population. Can the situation with EBOV serve as proof of principle for coping with other hemorrhagic fever outbreaks? We think so, at least for the closely related MARV, as the human-to-human transmission mechanism is the same. However, LASV and CCHFV spread via different routes, and lessons learned from the EBOV response might not apply to these pathogens. Nonetheless, a common theme emerges regarding unlicensed therapeutics and vaccines; it would be beneficial to have compassionate-use agreements in place in countries at risk for epidemics of these viruses. Several vaccine and therapeutic approaches use the same platforms for a variety of viruses, including those mentioned, and have demonstrated efficacy in preclinical studies. It would help immensely if standard protocols for known emerging virus outbreaks could be put in place to enable more rapid deployment of countermeasures to limit the spread of these deadly diseases.

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