Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Expert Rev Vaccines. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Expert Rev Vaccines. 2016 September ; 15(9): 1101–1112. doi:10.1080/14760584.2016.1187566.

Ebola Virus Disease Candidate Vaccines Under Evaluation in Clinical Trials

Karen A. Martins[1], **Peter B. Jahrling**[2], **Sina Bavari**[1], and **Jens H. Kuhn**[2]

[1]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA

[2]Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, USA

Summary

Filoviruses are the etiological agents of two human illnesses: Ebola virus disease and Marburg virus disease. Until 2013, medical countermeasure development against these afflictions was limited to only a few research institutes worldwide as both infections were considered exotic due to very low case numbers. Together with the high case-fatality rate of both diseases, evaluation of any candidate countermeasure in properly controlled clinical trials seemed impossible. However, in 2013, Ebola virus was identified as the etiological agent of a large disease outbreak in Western Africa including almost 30,000 infections and more than 11,000 deaths, including case exportations to Europe and North America. These large case numbers resulted in medical countermeasure development against Ebola virus disease becoming a global public-health priority. This review summarizes the status quo of candidate vaccines against Ebola virus disease, with a focus on those that are currently under evaluation in clinical trials.

Keywords

candidate vaccine; clinical trial; Ebola virus; Ebola virus disease; ebolavirus; EBOV; EVD; *Filoviridae*; filovirus; MCM; medical countermeasure; vaccine

1. INTRODUCTION

Filoviruses (the members of the mononegaviral family *Filoviridae*) cause two diseases recognized by the World Health Organization: Ebola virus disease (EVD) can be caused by Bundibugyo virus, Ebola virus (EBOV), Sudan virus (SUDV), and Taï Forest virus, whereas Marburg virus disease can be caused by Marburg virus (MARV) and Ravn virus [1]. Until 2013, filovirus infections were considered exotic infections that had little or no impact on

Address correspondence to: Jens H. Kuhn: Integrated Research Facility at Fort Detrick (IRF-Frederick), Division of Clinical Research (DCR), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), B-8200 Research Plaza, Fort Detrick, Frederick, MD 21702, USA; Phone: +1-301-631-7245; Fax: +1-301-631-7389; kuhnjens@mail.nih.gov.

Declaration of Interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

overall public health [2]. By the end of 2013, the total of all known human filovirus infections since 1967 reached 2,937, including 2,018 deaths (EVD: 2,460/1,633; Marburg virus disease: 477/385). EBOV-caused EVD amounted to 1,469 cases and 1,150 deaths [1]. However, at the end of 2013, an EBOV-caused EVD outbreak came to the forefront of primarily three Western African countries (Guinea, Liberia, and Sierra Leone), which, by its sheer magnitude, demonstrated that EBOV has the potential to cause large lethal epidemics that can threaten entire economies: by March 2016, this outbreak encompassed a staggering 28,646 human infections including 11,323 deaths (case-fatality rate = 39.53%) [3].

2. EBOLA VIRUS CANDIDATE VACCINE RESEARCH AND DEVELOPMENT

Development of vaccines for protection against infection with rare or exotic pathogens typically falls into the spheres of public health and/or biodefense. Such development does not, however, often pique the interest from the pharmaceutical industry. With little financial incentive to justify a private company's investment into vaccines that only few people would actually need, candidate vaccines for rare diseases often languish at the research bench stage, regardless of the strength of the preclinical studies assessing them. The 2013–2016 EVD outbreak in Western Africa however, was a shock to the system. EBOV candidate vaccines came roaring to the forefront in the popular press during this outbreak, demonstrating that a handful of research institutes had indeed been fully engaged in Ebola virus and other filovirus vaccine research for many years despite the then-low profile of the target agents. Prior to 2014, a total of four clinical trials that tested potential filovirus vaccines had been run in the US, all of them conducted by the National Institute of Allergy and Infectious Diseases (NIAID) of the US National Institutes of Health (NIH). Three of these candidate vaccines were based on a NIAID DNA-vaccine platform. The fourth, the NIAID Vaccine Research Center (VRC) rAd5-vectored vaccine VRC-EBOADV018-00-VP, would prove to be the precursor to one of the most promising anti-EBOV vaccines currently under development. Between 2014 and the time of writing, at least forty clinical trials are underway with more than eight different filovirus candidate vaccines. All of these express the filovirus glycoprotein ($GP_{1,2}$) as the primary immunogen, but utilize different platforms for expression [4]. This review will highlight the candidate vaccines currently under clinical investigation, with a brief discussion of promising preclinical candidates.

3. REPLICATION-COMPETENT, VECTORED VACCINES

3.1 Vesiculovirus Vectors

3.1.1 rVSV-ZEBOV—Vesicular stomatitis Indiana virus (VSIV) is a single-stranded, negative-sense RNA mononegavirus (*Mononegavirales*: *Rhabdoviridae*: *Vesiculovirus*) that typically infects livestock and rarely infects humans [5–7]. The VSIV genome has five genes that are expressed in a sequential and polar manner [8]. Using a reverse genetics system for VSIV in existence since 1995, a researcher can easily manipulate the virus and develop vaccine vectors [9]. Recombinant VSIV-based vaccines induce strong cellular and humoral immunity, are easily propagated *in vitro*, and undergo little, if any, genetic recombination or genetic reassortment [10]. Significant research efforts have aimed to develop methods of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

VSIV attenuation, at least partially for the purpose of using the resulting viruses as the basis for vaccine vectors [11].

The only EVD candidate vaccine for which human efficacy data exist, the "rVSV-ZEBOV" vaccine, is a replication competent VSIV-vectored vaccine [12–14]. This vaccine was developed by the Public Health Agency of Canada and licensed to NewLink Genetics and then Merck & Co. The rVSV-ZEBOV vaccine has been referred to as VSVG-ZEBOV-GP, rVSV-ZEBOV, VSV-ZEBOV, rVSVdeltaG-ZEBOV-GP, BPSC1001, and, most recently, V920 ["VSV" is a colloquial abbreviation for VSIV and "ZEBOV" is an outdated abbreviation for EBOV]. The distinguishing feature of rVSV-ZEBOV is that EBOV $GP_{1,2}$ replaces the VSIV glycoprotein (G), resulting in exclusive expression of EBOV $GP_{1,2}$ on virions produced from the recombinant VSIV backbone [12]. This glycoprotein exchange is anticipated to enhance the safety of the vaccine, as VSIV G is associated with neurovirulence and disease in infected animals [15,16].

According to the registry of clinical trials maintained by NIH (https://clinicaltrials.gov), rVSV-ZEBOV is or has been used in ten clinical trials, including trials in Liberia, Kenya, and Sierra Leone (accession numbers NCT02344407, NCT02296983, NCT02378753, respectively). Current efforts are focused on assessing lot consistency (NCT02503202) and examining the impact of escalating the vaccine dose (highest dose, 1×10^8 pfu) on immunogenicity. The most impactful of these trials, however, have been those conducted in Western Africa in collaboration between NIAID and the World Health Organization, specifically the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) trial and the Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE). In addition, the Guinea Ring Vaccination Trial, conducted under the auspices of the World Health Organization, was extended to Sierra Leone in August 2015 (http://www.afro.who.int/en/sierra-leone/press-materials/item/7962-guinea-ring-vaccination-trial-extended-to-sierra-leone-to-vaccinate-contacts-of-new-ebola-case.html) (Pan African Clinical Trials Registry, PACTR201503001057193).

The PREVAIL trial is an ongoing study to vaccinate individuals in Liberia with either rVSV-ZEBOV or the NIAID/Merck ChAd3-EBOZ vaccine (NCT02344407) [17] [EBOZ is another outdated abbreviation for EBOV]. PREVAIL is largely a safety study, which is performed with the intention to upgrade it to an efficacy study were an EVD outbreak to reemerge. STRIVE is also ongoing, but involves the development of rVSV-ZEBOV for frontline workers, including healthcare workers, ambulatory workers, and laboratory workers who may come in contact with EVD cases professionally (NCT02378753). The Guinea Ring Trial is the first EVD vaccine efficacy study. Contacts of cases with confirmed EBOV infection were enrolled in the trial and received rVSV-ZEBOV (2×10^7 pfu) either immediately or 21 days later. rVSV-ZEBOV was administered in clusters, such that all contacts of each index case received either the immediate or delayed vaccination. Approximately 40 clusters of individuals were enrolled in each arm. None of the individuals receiving the vaccine immediately developed EVD. In contrast, 16 cases of EBOV infection occurred in seven different clusters in the delayed vaccination group, suggesting that the vaccine was efficacious if given early in the disease course [18].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

While the efficacy associated with rVSV-ZEBOV is highly encouraging, the vaccine has been marred by safety concerns. In the Guinea Ring Study, 43 serious adverse events were reported and are still under investigation; only one serious adverse event, an episode of febrile illness, has been causally linked to the vaccination at this time [18]. Additionally, adverse events raised concerns about the safety profile of the vaccine used in European clinical trials [19]. In contrast, safety studies in immunocompromised nonhuman primates had suggested that the vaccine would be safe in special populations [20]. In other multiple clinical studies in the US, Africa, and Europe, significant adverse events with rVSV-ZEBOV have not been observed [21,22]. As a direct relationship between vaccine dose and both immunogenicity (as evaluated by antibody titers) and safety (as evaluated by adverse events) is apparent, an acceptable balance between immunogenicity and safety should be determined based on collected clinical data [19]. Antibody titer data from protected individuals in the Guinea Ring Trial may aid in determining optimal antibody titers desired for protection.

3.1.2 In the pipeline—In contrast to rVSV-ZEBOV, VesiculoVax (Profectus Biosciences) is based on a recombinant VSIV that expresses both VSIV G and EBOV $GP_{1,2}$. However, a rearrangement of the gene order in the recombinant backbone results in preferential expression of EBOV $GP_{1,2}$ and minimal expression of VSIV G [23,24]. Potential benefits of this candidate vaccine are that the rearrangement of genes also places the VSIV nucleocapsid (N) gene in the fourth position (rather than the wild-type first position), which attenuates the virus [11]. Recently, researchers from Profectus Biosciences demonstrated that VesiculoVax is efficacious in nonhuman primates against infection with EBOV Makona-C07, which is an isolate obtained during the 2013–2016 Western Africa EVD outbreak [23]. VesiculoVax is produced with $GP_{1,2}$ from the EBOV Yambuku-Mayinga isolate obtained in 1976, as are many of the candidate vaccines discussed here. Consequently, the VesiculoVax nonhuman-primate study demonstrates the ability of the vaccine to cross-protect against different EBOV variants to some extent. Researchers from Profectus Biosciences suggest that VesiculoVax may be safer than other VSIV platforms due to the incorporated attenuation, and a head-to-head comparison between rVSV-ZEBOV and VesiculoVax candidates may therefore be of interest. The VesiculoVax platform has successfully entered a clinical trial with an antigen insert (human immunodeficiency virus-1 [HIV-1]) other than EBOV, and therefore theoretically should be poised to transition to the clinic if funding were provided (NCT01438606).

3.2 Respirovirus Vectors

Respiroviruses (*Mononegavirales*: *Paramyxoviridae*: *Respirovirus*) are single-stranded, negative-sense, RNA viruses that are actively being explored as potential vaccine vectors. Early studies examining the utility of recombinant human parainfluenza virus 3 (HPIV-3) as a vaccine vector demonstrated that multiple glycoproteins could be inserted into the viral backbone. The utility of insertion was impacted by the number and position of insertions and by whether the resulting protein was incorporated into packaged virions [25]. Potential benefits of HPIV-3 as a vector include its genomic stability, ease of production, and the efficiency with which foreign inserts are expressed [26,27]. However, as for adenovirus platforms, pre-existing immunity to HPIV-3 may reduce vaccine efficacy. Preclinical efforts are underway to circumvent the issues of pre-existing immunity [28,29].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3.2.1 HPIV3-EBOVZ GP—Results from preclinical studies in HPIV-3-immune guinea pigs with EBOV $GP_{1,2}$-expressing HPIV-3 have suggested that while pre-existing immunity to the vector suppressed vector replication, the animals nonetheless mounted a robust immune response to the EBOV $GP_{1,2}$ [30]. In contrast, in nonhuman primates, low levels of vector replication were detectable despite pre-existing immunity to HPIV-3 [31]. While immunogenicity was initially reduced in vector-immune monkeys, the IgG, IgA, and neutralizing antibody titers to EBOV $GP_{1,2}$ were essentially equivalent to those of vector-naïve monkeys after two vaccinations [31]. Results from a subsequent nonhuman-primate study demonstrated that respiratory or intranasal/intratracheal administration of HPIV-3/EBOVZ GP also elicited robust immune responses and provided protection from disease after intramuscular exposure to EBOV [32].

NIAID is sponsoring clinical trials with an HPIV-3-based vaccine, “HPIV3-EbovZ GP” (NCT02564575). Perhaps the most unique and intriguing characteristic of this candidate vaccine is that the vaccine is administered intranasally; all other vaccines currently in clinical trials are administered intramuscularly. The potential benefits of intranasal administration are the ease of administration as well as the effective induction of mucosal immunity. Mucosal immunity may be of particular importance in filovirus disease outbreaks during which the vast majority of infections are thought to occur through mucosal exposure to contaminated bodily fluids. The current Phase I study is examining two doses of HPIV3-EbovZ GP administered twice at a 4–8 week-interval. Viremia, the duration of HPIV-3 shedding, and safety will be assessed in the volunteers.

4. EPLICATION-INCOMPETENT, VECTORED VACCINES

4.1 Adenovirus vectors

Adenoviruses (*Adenoviridae*) have been developed as vaccine vectors for multiple antigens, but pre-existing immunity to the selected adenovirus may impact vaccine efficacy [33–35]. Vaccines based on adenovirus backbones may be ineffective if a patient’s pre-existing immunity to the adenovirus stifles the immune response to the intended target antigen [33]. Moreover, pre-existing immunity to the adenovirus 5 (Ad5; species *Human mastadenovirus C*) backbone actually seemed to have a negative effect on prognosis in an HIV-1/AIDS vaccine trial [36,37]. Nonetheless, one of the first EVD vaccines to enter clinical trials was, in fact, an adenovirus-based vaccine targeting EBOV (VRC-EBOADV018-00-VP) [38,39]. Subsequent research has examined the properties of various adenovirus vectors on vaccine immunogenicity and efficacy, indicating that concerns about adenovirus-based vaccines can be mitigated by careful selection of the backbone virus [40,41]. Two of the current, advanced EVD candidate vaccines that are based on adenoviruses circumvent the issue of pre-existing immunity either by enlisting a chimpanzee adenovirus 3 (ChAd3; species *Human mastadenovirus C*) vector or a vetted human vector against which the population at large has limited pre-existing immunity. Additionally, pre-existing immunity to adenoviruses may be abrogated by administering the vaccine orally, a route that has been pursued by some vaccine developers [42–45].

Three different adenovirus-based vaccines are currently under clinical investigation. The first vaccine is based on ChAd3. This vaccine was developed by the NIAID VRC and is in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

multiple Phase II clinical trials (NCT02485301, NCT02548078, NCT02344407, NCT02289027). The vaccine is almost universally administered as a heterologous prime-boost vaccination regimen with a modified vaccinia virus Ankara (MVA)-based vaccine. The second candidate is a vaccine based on adenovirus 26 (Ad26, species *Human mastadenovirus D*), developed by Crucell Holland B.V., a subsidiary of Johnson and Johnson. Selection of the Ad26 backbone was likely based on studies comparing the immunogenicity of Ad35 (species *Human mastadenovirus B*), Ad26, and Ad5 backbones in nonhuman primates [40]. Ad35 was studied because of very low seroprevalence of antibodies against Ad35 in the human population whereas Ad26 was studied because it elicits low levels of neutralizing antibodies. Induction of high levels of neutralizing antibodies was thought to be the major drawback for the Ad5-based vaccine platform [40,46,47]. The third candidate vaccine is based on Ad5 and is sponsored by the Jiāngsū Provincial Centers for Disease Control and Prevention in China (NCT02326194, NCT02575456, NCT02533791). All of these adenovirus backbones were tailored to become replication-incompetent by deleting the E1 regions [48,49]. EBOV $GP_{1,2}$ is encoded by the adenovirus backbone and is therefore expressed upon entry of a target cell by the encoded adenovirion; $GP_{1,2}$ is not located on the adenovirus particle surface as in the case of VSIV-based vaccines[33].

4.1.1 ChAd3-EBOZ—The ChAd3 backbone is an effective antigen delivery system and has been tested as a candidate vaccine against multiple pathogens [50,51]. Two separate ChAd3-based filovirus vaccines were developed by the NIAID VRC. VRC-EBOADC069-00-VP (ChAd3-EBO) expresses both the EBOV and the related Sudan virus (SUDV) $GP_{1,2}$s in two separate vectors, mixed at a 1:1 ratio. VRC-EBOADC076-00-VP (ChAd3-EBOZ), in contrast, only includes EBOV $GP_{1,2}$. Both candidate vaccines have been evaluated in Phase I studies. ChAd3-EBOZ has moved on to Phase II trials and the Phase III PREVAIL trial, and the vaccine is licensed by GlaxoSmithKline (GSK) as GSK3390107A.

ChAd3-EBOZ was among the first EVD candidate vaccines evaluated for duration of immunity in nonhuman primates. Investigators found that immunity waned in crab-eating macaques after a single vaccination and therefore included a booster vaccination with an MVA-vectored vaccine [52]. Potentially stemming from these data and the immunogenicity data collected during an early Phase I trial [53], several ongoing clinical trials are utilizing a heterologous prime-boost with ChAd3-EBOZ and MVA-based vaccines. An MVA-filovirus vaccine produced by Bavarian Nordic (MVA-BN-Filo) has been included in all heterologous vaccination studies initiated in 2014 or earlier. Starting in 2015, however, there has been a switch to using an MVA vaccine developed by the NIAID VRC, VRC-EBOMVA079-00-VP (MVA-EbolaZ).

Current clinical studies are evaluating the impact of timing of the boost vaccination, directly comparing administration of the boost (MVA-EBOZ) at days 14 and 28 and even as early as day 7 (NCT02451891, NCT02240875). Moreover, different combinations of the two candidate vaccines (ChAd3-EBOZ, MVA-EbolaZ) and different dose levels are being evaluated to determine the most immunogenic combination. Investigators from a recently published Phase I study performed in Mali and the US suggested that ChAd3-EBOZ may be adequate for short-term protection, as, for example, in a ring vaccination setting. However, a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

boost with an MVA vaccine would likely provide more durable protection [54]. Notably, the highest ChAd3-EBOZ dose tested, 10^{11} particle units (pu), was selected as the optimal dose level [54].

ChAd3-EBOZ is currently being evaluated in clinical trials in Africa, including in Mali, Senegal, Uganda, and Liberia (NCT02368119, NCT02485301, NCT02548078, NCT02267109, NCT02485912, NCT02354404, NCT02344407). Critically, ChAd3-EBOZ is the comparator treatment arm to rVSV-ZEBOV treatment arm in the PREVAIL study; data from the PREVAIL study that directly compare the immunogenicity of the two vaccines will be of great interest to the filovirus vaccine community. Historically, the adenovirus-based vaccine was thought to rely on a CD8 T-cell response for maximum efficacy, whereas a humoral response is thought to be the primary efficacy correlate of rVSV-ZEBOV [55–57].

As a non-replicating vaccine, ChAd3-EBOZ has not caused the concerning adverse events seen with rVSV-ZEBOV; however, ChAd3-EBOZ also appears to be inadequately immunogenic as a single vaccination [53,54,58]. In some EVD outbreak situations, administration of a booster vaccination may not be feasible and will certainly be more costly than an efficacious single vaccination. Moreover, adenovirus based vaccines will not only require multiple vaccinations, but will require heterologous vaccinations. The immune response mounted to the adenovirus upon primary vaccination will reduce uptake of the adenovirus vaccine, reducing expression of the antigen, and therefore reducing or eliminating the immune response to the antigen [39,59]. Inadequate immunogenicity of a homologous adenovirus prime/boost regimen was indeed observed in nonhuman primate studies with ChAd3-EBOZ, and makes heterologous prime/boost vaccination a requirement for durable protection with this vaccine [52]. Overall, ChAd3-EBOZ appears to be an efficacious and safe candidate vaccine, but the logistical complications associated with administering a heterologous boost may reduce its impact in endemic regions.

4.1.2 Ad26.ZEBOV—Crucell Holland B.V. developed the Ad26-vectored EVD vaccine Ad26.ZEBOV based on extensive experience testing Ad26 and Ad35 vectors for malaria and filovirus disease vaccinations [60–63]. These early data, in combination with comparison of these virus vectors to Ad5, likely led Crucell Holland B.V. to select Ad26 for advanced vaccine development [40].

Possibly due to similar restrictions in immunogenicity and homologous boosting as those associated with the ChAd3-EBOZ, nearly all clinical studies conducted with Ad26.ZEBOV are being performed in the context of heterologous boosts with an MVA-vectored vaccine (MVA-BN-Filo). Due to the recognition that either adenovirus candidate vaccine will require an MVA or comparable pairing, a clinical trial sponsored by the University of Oxford is underway to directly compare the ChAd3-EBOZ and Ad26.ZEBOV (NCT02495246). The study compares priming and boosting with either ChAd3-EBOZ or Ad26.ZEBOV, administered at either 28 or 56 days post-priming. Data from this study may suggest which candidate vaccine should be advanced or whether a heterotypic adenovirus vaccination regimen would be effective.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

In parallel with this comparative study, other Ad26.ZEBOV/MVA-BN-Filo clinical studies are focused on determining which candidate should be prime vs. boost, as well as on dose level and vaccination schedule. Data from one of these studies have been published shortly before this review went to press [64], indicating that priming with Ad26.ZEBOV may be preferable to priming with MVA-BN-Filo. No severe adverse effects were reported in this study. Multiple studies are underway or have been completed in Europe and Africa, including sites in Tanzania, Uganda, Kenya, and Sierra Leone. Additionally, Ad26.ZEBOV/MVA-BN-Filo vaccination is being studied in children, the elderly, and immune compromised individuals (NCT02564523, NCT02661464, NCT02509494). Ad26.ZEBOV has a strong safety profile but the efficacy of this candidate vaccine may be hampered by significant background immunity to the vector in potential recipients. As with many of these vaccine candidates, the low quantity of peer-reviewed publications on the products does not reflect the number of clinical trials completed or ongoing. Presumably, a significant amount of accumulated data will become available within the next year, but for now one must unfortunately rely on (potentially biased) company reports. For the Ad26.ZEBOV/MVA-BN-Filo vaccine combination, Janssen has created a project called EBOVAC (http://www.ebovac.org/), which apparently serves to collate and present data acquired from the numerous ongoing clinical trials of EVD candidate vaccines.

4.1.3 Ad5-EBOV—Despite concerns regarding Ad5-vectored vaccines, one Ad5-based EVD vaccine (Ad5-EBOV) is under clinical investigation both in China and in Sierra Leone. The vaccine is sponsored by the Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc. and features EBOV $GP_{1,2}$ encoded by the Ad5 backbone. One unique attribute of this vaccine is the use of a glycoprotein gene encoding $GP_{1,2}$ of the EBOV Makona-C15 isolate rather than EBOV Yambuku-Mayinga, with the aim to increase efficacy against the 2013–2016 Western Africa EBOV variant [65]. Investigators assessed pre-existing immunity of volunteers to the Ad5 backbone and found that a majority did indeed have antibodies against Ad5 [65]. The investigators also evaluated anti-EBOV $GP_{1,2}$ titers after vaccination, comparing the titers of individuals with high vs. low pre-existing immunity to Ad5. Pre-existing immunity to Ad5 reduced anti-EBOV $GP_{1,2}$ antibody titers significantly, but administration of a higher vaccine dose could potentially ameliorate this reduction [65]. Considering that Ad5 immunity is quite prevalent in the African population, the use of Ad5-EBOV will only be advocated if immunogenicity concerns in the endemic population are overcome [66]. A Phase 2 study is now underway in Sierra Leone to compare the efficacy of two vaccine dosages (NCT02575456).

4.1.4 In the pipeline—The development of an orally or intranasally administered Ad5-vectored EVD vaccine has been pursued [67,68], and a recent study suggests that sublingual administration of an adenovirus-based vaccine may provide durable protection at least in nonhuman primates [69]. Oral administration of a live adenovirus-vectored vaccine abrogates the relevance of pre-existing vector immunity, resulting in a robust immune response to the delivered antigen [42,43,70,71]. The US company Vaxart, which is currently evaluating an oral adenovirus-based influenza vaccine in a Phase I clinical trial (NCT02547792), has also developed an oral adenovirus-based EVD vaccine with plans to enter clinical trials.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

5. Poxvirus Vectors

MVA is a replication-incompetent, attenuated vector developed from vaccinia virus (*Poxviridae*: *Chordopoxvirinae*: *Orthopoxvirus*) [72]. Antigens encoded by the vector are expressed in host target cells upon infection [72]. MVA is itself stockpiled as a potential vaccine against smallpox (caused by the closely related variola virus), but it has also been used as a vector in the development of vaccines against many diseases, including malaria, hepatitis C, influenza, and, of course, filovirus diseases [50,73,74].

MVA-vectored vaccines have been used as heterologous boosts in multiple clinical trials, generally in combination with an adenovirus-based vaccine [75]. Bavarian Nordic's MVA-BN-Filo has been combined with both ChAd3-EBOZ and Ad26.ZEBOV. However, formation of an Ebola Vaccine Development Consortium last year, which includes Bavarian Nordic and Johnson & Johnson, may have solidified the Ad26.ZEBOV collaboration with MVA-BN-Filo (https://www.jnj.com/news/all/Johnson-Johnson-Announces-Formation-of-Ebola-Vaccine-Development-Consortia-Gains-Funding-from-Innovative-Medicines-Initiative). Around the same timeframe, NIAID developed its own MVA-EbolaZ (VRC-EBOMVA079-00-VP), which has been used in more recent ChAd3-EBOZ clinical trials.

5.1 MVA-BN-Filo

Bavarian Nordic's MVA-BN-Filo vaccine encodes four filovirus proteins, EBOV $GP_{1,2}$, SUDV $GP_{1,2}$, Marburg virus (MARV) $GP_{1,2}$, and Taï Forest virus (TAFV) nucleoprotein (NP). MVA-BN-Filo has the potential, therefore, to induce immunity against multiple filoviruses. MVA-BN-Filo significantly enhanced T-cell responses when administered as a boost after a ChAd3-EBOZ prime [54]. Additionally, inclusion of an MVA-BN-Filo boost resulted in a significant increase of anti-filovirus antibody titers, with a geometric mean-fold increase of 26. Interestingly, individuals receiving the MVA-BN-Filo boost did not consistently mount antibody responses to SUDV or MARV $GP_{1,2}$ [54].

Few data have been published for MVA-BN-Filo alone, as this vaccine was developed specifically in response to the 2013–2016 Western Africa EVD outbreak. Bavarian Nordic has actively pursued the inclusion of adjuvants, including double-stranded RNA, into their vaccine platform in the past, but it is unclear whether these efforts will extend to the filovirus products [76]. Current clinical trials are examining the possibility of including MVA-BN-Filo as a prime as well as a boost with the Ad26.ZEBOV partner vaccine (NCT02376400, NCT02325050). The combinatorial vaccine regimen is currently being evaluated in clinical trials at multiple sites in Africa, the US, and Europe. Janssen's EBOVAC project will presumably be summarizing and releasing data as they become available.

5.2 MVA-EbolaZ

NIAID's VRC has developed an MVA-based vaccine called MVA-EbolaZ (VRC-EBOMVA079-00-VP). Preclinical data have not been published for this vaccine, but it has been paired with ChAd3-EBOZ in recent clinical trials. This MVA-based vaccine expresses only EBOV (Yambuku-Mayinga) $GP_{1,2}$. Preclinical work by VRC researchers with an MVA vector expressing both EBOV and SUDV $GP_{1,2}$ showed that the MVA vaccine alone elicited

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

a poor antibody response in nonhuman primates [52]. However, administering the MVA vaccine as a boost after a ChAd3-EBOZ prime resulted in 100% survival and enhanced immune responses, laying the groundwork for subsequent clinical studies [52].

6. DNA VACCINES

DNA vaccines are inexpensive, safe, and easy to produce; however, the pitfall of the platform is that DNA vaccines are often poorly immunogenic by intramuscular administration. Electroporation is currently used to deliver many DNA candidate vaccines into the nucleus of the target cells, which is required for effective antigen production and therefore immunogenicity. When administered properly, DNA vaccines elicit strong T-cell responses and are potentially effective for protection from viral infections. Two research groups have EBOV DNA vaccines that currently are or have been in clinical trials: NIAID and Inovio.

While the safety profile, cost effectiveness, and ease of production make DNA vaccines attractive candidates, the difficulty in vaccine administration is a serious hurdle to their licensure. It is impractical to deploy hundreds of electroporation instruments to remote areas in Africa, where instrument failure or poor maintenance could mean that the vaccine cannot be administered. Nonetheless, the possibility that a more efficient delivery technology will be developed would make DNA vaccines more practical. Moreover, if vaccination of only a small number of people were required to perhaps ring-vaccinate to contain a new EVD outbreak, DNA vaccines offer an easily amenable platform that could be made variant-specific and administered effectively to a small number of at-risk individuals.

7. NIAID-Sponsored DNA vaccines (VRC-EBODNA023-00-VP, VRCEBODNA012-00-VP, and VRCMARDNA025-00-VP)

NIAID sponsored three clinical trials for their DNA candidate vaccines between 2003 and 2013. Two of these trials were Phase 1 trials in the US, whereas the third was conducted in Uganda. The vaccines in all studies were administered by Biojector, a needle-free injection system.

The first trial tested an ebolavirus DNA vaccine (VRCEBODNA012-00-VP) that included three plasmids expressing EBOV $GP_{1,2}$, SUDV $GP_{1,2}$, and EBOV NP. The expressed $GP_{1,2s}$ lacked their transmembrane domains in an effort to increase release of the proteins from the expressing-cell membrane after expression [77]. VRCEBODNA012-00-VP was administered as part of a prime-boost regimen with an early Ad5-based vaccine as the boost, also produced by NIAID. The impetus for these studies was a nonhuman primate study wherein three vaccinations with the DNA vaccine, followed by a single vaccination with the Ad5 vaccine, resulted in protection from EBOV infection [49]. After vaccination, all volunteers had seroconverted to at least one of the antigens [77].

A second generation DNA vaccine, VRC-EBODNA023-00-VP, included two plasmids expressing full-length EBOV and SUDV $GP_{1,2}$, and it was tested in parallel with a DNA vaccine for Marburg virus disease, VRCMARDNA025-00-VP, which expressed full-length

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

MARV $GP_{1,2}$ [78]. After three vaccinations, only 56% of subjects had seroconverted to the Ebola virus $GP_{1,2}$ and 89% to the Sudan virus $GP_{1,2}$, suggesting that the DNA vaccines had not been administered effectively [78]. The potential impact of a heterologous boost on immunogenicity was not evaluated. Because the safety data from the study were reassuring, these vaccines went on to be evaluated in a Phase 1b trial in Uganda. Volunteers received 4 mg of EBOV DNA vaccine, MARV DNA vaccine, or both (NCT00997607) using Biojector 2000. While the safety profile of the vaccine was acceptable, even after three vaccinations the immune response of volunteers was poor, and antibody titers had nearly returned to baseline levels by 44 weeks after vaccination [79].

8. Inovio-developed DNA Vaccines (INO-4201, INO-4202, and INO-4212)

Inovio has developed three DNA vaccines: INO-4201 expresses EBOV $GP_{1,2}$ from pre-2013 EBOV variants, INO-4202 expresses EBOV Makona $GP_{1,2}$, and INO-4212 is a one-to-one mixture of INO-4201 and INO-4202. In addition, Inovio is testing the impact of including a DNA vaccine encoding IL-12 (INO-9012), which may help enhance the immune response to vaccination. Administration of the vaccines will be intramuscular, but the injection will be followed by electroporation (NCT02464670) [80]. Data from these studies should become available in late 2016.

9. SUBUNIT VACCINES

It has long been known that ebolavirus $GP_{1,2}$ is the required antigen for obtaining protective immunity [28,81,82]. All of the vaccines in clinical trials are using $GP_{1,2}$ as their antigen, and measurement of $GP_{1,2}$-specific immune responses will likely be the ultimate correlate of protection. Naturally, investigators have therefore looked at the potential utility of protein-based and subunit vaccines. A $GP_{1,2}$-based subunit vaccine would face the hurdles of immunogenicity and manufacturing. Protein-based vaccines lack “danger signals” and typically are poorly immunogenic in the absence of a vaccine adjuvant. In addition, $GP_{1,2}$ is a notoriously difficult protein to produce and, due to its cytotoxicity, cell lines producing $GP_{1,2}$ are also difficult to maintain. Despite these obstacles, one protein-based platform has advanced into Phase 1 clinical trials (NCT02370589).

9.1 Ebola virus $GP_{1,2}$ with Matrix-M adjuvant

Novavax has produced an EBOV Makona $GP_{1,2}$ nanoparticle vaccine that is adjuvanted with proprietary Matrix-M. Matrix-M, according to Novavax, is a phospholipid base with synthetic cholesterol and saponin. There are multiple vaccine adjuvants that are immune-stimulating complexes (ISCOMs) like Matrix-M, and this class of adjuvants is associated with eliciting strong humoral and cellular responses [83]. To produce the vaccine, Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV; *Baculoviridae*: *Alphabaculovirus*) expressing the antigen of interest, in this case EBOV $GP_{1,2}$, is used to infect *Spodoptera frugiperda* Sf9 insect cells. $GP_{1,2}$ is then expressed on the surface of infected cells. The cells are lysed, and the $GP_{1,2}$ is collected for formulation with the adjuvant. Primary publications on this product have not yet been released, but a similar approach has been used by Novavax for its human respiratory syncytial virus vaccine products [84]. Data from the ongoing Phase 1 trial should become available soon.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

9.2 In the pipeline

The US Army Medical Research Institute of Infectious Disease (USAMRIID) developed a filovirion-like particle more than twenty years ago [82,85–90]. These virion- or ("virus")-like particles (VLPs) have alternately been produced in Sf9 cells via a baculovirus system, much like the Novavax product, or in human 293 cell lines. VLPs have been shown to be highly immunogenic and confer protection in the absence of adjuvants to rodents exposed to rodent-adapted EBOV [91,92]. Inclusion of adjuvant, however, results in significant dose sparing and is required for achieving protection in nonhuman primates [93–97]. The VLP platform was anticipated to move forward to clinical trials, but manufacturing issues have hampered its progression.

10. CONCLUSIONS

In this review, we refrained from delving too deeply into immunogenicity data collected from the various past and ongoing vaccine trials due to the lack of standardized assays by which samples from different studies can be compared. Efforts are underway to standardize an enzyme-linked immunosorbent assay for antibody titer evaluation, as well as an assay to evaluate neutralizing antibody titers [98,99]. Vaccine development would benefit considerably from agreement by the various sponsors to have an independent organization run comparative immunological assays with their study samples.

For deployment to regions affected by filovirus infection, cold-chain requirements should be considered. These requirements are difficult to adhere to in parts of Africa, where supply of electricity and therefore refrigeration is not reliable. Vaccines that are effective after lyophilization or storage at room temperature may provide a competitive benefit over those that require colder storage temperatures. While pre-existing immunity to the Ad5 vector may affect the efficacy of the vaccine, use of Ad5-EBOV in a lyophilized format is at least suggestive that adenovirus-based vaccines can be efficacious after lyophilization [65]. Other vaccines discussed here are undergoing stability studies or have been demonstrated to be stable at refrigeration conditions.

Ebola virus and other filoviruses cause severe disease with a rapid onset. The development of a vaccine rapidly inducing immunity via a single vaccination would be ideal. The requirement of heterologous boosts for the adenovirus-based vaccines is a drawback in terms of production but also in terms of practical administration. While a single vaccination would be ideal, even a homologous boost would be preferable to a heterologous boost. In this area, VSIV-based vaccines are promising.

A third point to consider in developing an EVD vaccine for an African population is that the microbiome, nutritional status, and pre-existing immunity of vaccinees are quite distinct from those of the European or American populations. These differences between the developed world and the developing world may impact vaccine efficacy. Thus, while an orally administered vaccine would seem to be ideal for its ease of administration and lack of cold-chain requirements, there is evidence that oral vaccines fail in populations with disturbed microbiota, poor nutrition, and high intestinal inflammation [100–102]. Additionally, developers of vectored vaccines should consider the pre-existing immunity of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the target population, which may differ from that of the country in which the product is developed.

Finally, the vast majority of EVD vaccines currently evaluated in clinical trials are administered intramuscularly; the single exception is HPIV3-EbovZ GP. Little research has been performed to ascertain whether intramuscular vaccine administration of a vaccine will protect against mucosal exposure, which is the most likely route of exposure to a filovirus during a natural disease outbreak. rVSV-ZEBOV administration in nonhuman primates via intranasal and oral administration has been compared to intramuscular administration [103]. Animals survived intramuscular exposure to EBOV regardless of vaccination route, and intranasal administration elicited higher peripheral IgA and IgG titers compared to that observed with intramuscular administration. Unfortunately, IgA titers in mucosal tissues were not evaluated, nor was protection from mucosal infection. An earlier nonhuman primate study using VSIV-vectored Marburg virus or EBOV $GP_{1,2}$, however, demonstrated vaccine efficacy against respiratory exposure to homologous virus [104]. Nonhuman primate studies using adenovirus-vectored filovirus vaccines have also demonstrated protection against respiratory exposure but data with the current clinical candidates are not yet available [105].

11. EXPERT COMMENTARY

The variety of EBOV vaccines currently in clinical trials is astounding considering the state of EBOV vaccine research only 5 or even only 3 years ago. The candidate vaccines currently under clinical investigation are based on multiple platforms and have varying benefits and pitfalls. From the current candidate vaccines, more than one candidate will likely have an acceptable safety record allowing advance to product licensure. Certainly there are benefits to advancing more than one candidate, as different vaccines may be more valuable in some settings than in others. Vaccines for an emerging outbreak should be fast acting and easily disseminated to remote regions. In contrast, multiple vaccine boosts might be acceptable for healthcare workers or military personnel if the vaccine is administered well in advance of deployment to an outbreak region. From a biodefense standpoint, protecting a target population from respiratory EBOV infection is desirable. A specialized vaccine for enhanced mucosal immune protection may be preferable for at-risk personnel.

For a re-emerging pathogen like EBOV, researchers have a unique opportunity to develop the best possible vaccine. If history is any indication, several years will pass before the next major EVD outbreak occurs. During that time, researchers could harness the knowledge gained from the current clinical candidates and, in collaboration with the institutes and agencies that developed them, work to develop an optimized vaccine candidate. Potentially, the next filovirus disease outbreak may not be due EBOV, but could be due to another known or unknown filovirus that will require the rapid production of a new vaccine. To that end, optimization of the vaccine platform for rapid modification of the expressed antigen would be a valuable research aim for investigators.

While one hopes that the next filovirus disease outbreak is years away, we could conceivably see an increase in the incidence of filovirus disease. As urban communities continue to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

expand and villages grow, we are increasingly encroaching on the territory of animals and other organisms that could harbor human pathogens. Naturally, our risk of exposure will increase. For the vaccine field in general, the impetus should be on developing vaccine platforms that can be rapidly modified to counter emerging threats.

12. FIVE-YEAR VIEW

The advancement of EBOV vaccines accelerated considerably during the recent Western Africa EVD outbreak, and clinical trials focused on safety and immunogenicity continue in Africa and the Western World. Several of these candidates will likely have an acceptable safety profile. Concurrently, efforts will proceed for improving these vaccines. These efforts may include increasing the attenuation and therefore safety of the VSIV vector; improving the immunogenicity of the MVA and adenovirus vectors, and subunit or protein based vaccines through adjuvants and novel delivery systems; and developing a less complicated device for DNA vaccine administration. Critically, testing of cold-chain requirements and the ability to lyophilize vaccine products will continue, and investigators will examine the ability of their vaccines to elicit mucosal immunity, regardless of vaccination route. The licensure of a vaccine will require either that animal data are accepted in place of human efficacy data or that clinical studies will be initiated during another filovirus disease outbreak. To enable this latter aim, clinical sites should be developed in Africa, which can be utilized for Phase 1 trials in the absence of an outbreak and for Phase 3 trials in the event of an outbreak. Relations between Western and African researchers and institutes may have been strengthened through collaborations during the recent Western African EVD outbreak, increasing the global sharing of information. Such relationships could potentially advance the scientific, clinical, and research capabilities of African countries. In addition, these relationships may contribute toward the Western World's understanding of endemic emerging infectious diseases, in which African researchers often have expertise.

Acknowledgments

K Martins and S Bavari are supported by funding from the US Department of Defense and the Defense Threat Reduction Agency (CB10208). The content of this publication does not necessarily reflect the views or policies of the US Department of the Army, the US Department of Defense, the US Department of Health and Human Services (DHHS) or of the institutions and companies affiliated with the authors. Jens H. Kuhn and this work were supported in part through Battelle Memorial Institute's prime contract with NIAID under Contract No. HHSN272200700016I. A subcontractor to Battelle Memorial Institute who performed this work is Jens H. Kuhn, an employee of Tunnell Government Services, Inc.

We thank Laura Bollinger (Integrated Research Facility at Fort Detrick) for providing Technical Writing Services in critically editing this manuscript on behalf of Battelle Memorial Institute through Battelle Memorial Institute's prime contract with NIAID under Contract No. HHSN272200700016I.

REFERENCES

Papers of special note have been highlighted as:

* of interest

** of considerable interest

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. Kuhn, JH. Ebolavirus and Marburgvirus Infections. In: Kasper, DL.; Fauci, AS.; Hauser, SL.; Longo, DL.; Jameson, JL.; Loscalzo, J., editors. Harrison's Principles of Internal Medicine. Columbus, Ohio, USA: McGraw-Hill Education; 2015. p. 1323-1329.
2. Geisbert TW, Jahrling PB. Exotic emerging viral diseases: progress and challenges. Nat Med. 2004; 10(12 Suppl):S110–S121. [PubMed: 15577929]
3. World Health Organization. Ebola Situation Reports. 2016. http://apps.who.int/ebola/ebola-situation-reports
4. Yang ZY, Duckers HJ, Sullivan NJ, et al. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nat Med. 2000; 6(8):886–889. [PubMed: 10932225]
5. Hanson RP. The natural history of vesicular stomatitis. Bacteriol Rev. 1952; 16(3):179–204. [PubMed: 12987386]
6. Huang AS, Wagner RR. Comparative sedimentation coefficients of RNA extracted from plaque-forming and defective particles of vesicular stomatitis virus. J Mol Biol. 1966; 22(2):381–384. [PubMed: 4291446]
7. Prevec L, Whitmore GF. Purification of vesicular stomatitis virus and the analysis of P^{32}-labeled viral components. Virology. 1963; 20:464–471. [PubMed: 14043410]
8. Ball LA, White CN. Order of transcription of genes of vesicular stomatitis virus. Proc Natl Acad Sci U S A. 1976; 73(2):442–446. [PubMed: 174107]
9. Lawson ND, Stillman EA, Whitt MA, Rose JK. Recombinant vesicular stomatitis viruses from DNA. Proc Natl Acad Sci U S A. 1995; 92(10):4477–4481. [PubMed: 7753828]
10. Geisbert TW, Feldmann H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. J Infect Dis. 2011; 204(Suppl 3):S1075–S1081. [PubMed: 21987744]
11. Clarke DK, Nasar F, Lee M, et al. Synergistic attenuation of vesicular stomatitis virus by combination of specific G gene truncations and N gene translocations. J Virol. 2007; 81(4):2056–2064. [PubMed: 17151112]
12. Jones SM, Feldmann H, Ströher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nat Med. 2005; 11(7):786–790. [PubMed: 15937495] ** First study demonstrating efficacy of a vesicular stomatitis Indiana virus vaccine in nonhuman primates.
13. Garbutt M, Liebscher R, Wahl-Jensen V, et al. Properties of replication-competent vesicular stomatitis virus vectors expressing glycoproteins of filoviruses and arenaviruses. J Virol. 2004; 78(10):5458–5465. [PubMed: 15113924]
14. Medaglini D, Harandi AM, Ottenhoff TH, et al. Ebola vaccine R&D: Filling the knowledge gaps. Sci Transl Med. 2015; 7(317):317ps324.
15. Martinez I, Rodriguez LL, Jimenez C, et al. Vesicular stomatitis virus glycoprotein is a determinant of pathogenesis in swine, a natural host. J Virol. 2003; 77(14):8039–8047. [PubMed: 12829843]
16. Publicover J, Ramsburg E, Rose JK. Characterization of nonpathogenic, live, viral vaccine vectors inducing potent cellular immune responses. J Virol. 2004; 78(17):9317–9324. [PubMed: 15308726]
17. Kennedy SB, Neaton JD, Lane HC, et al. Implementation of an Ebola virus disease vaccine clinical trial during the Ebola epidemic in Liberia: Design, procedures, and challenges. Clin Trials. 2016; 13(1):49–56. [PubMed: 26768572]
18. Henao-Restrepo AM, Longini IM, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet. 2015; 386(9996):857–866. [PubMed: 26248676] ** First efficacy trial of an Ebola virus vaccine.
19. Huttner A, Dayer JA, Yerly S, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. Lancet Infect Dis. 2015; 15(10):1156–1166. [PubMed: 26248510]
20. Geisbert TW, Daddario-Dicaprio KM, Lewis MG, et al. Vesicular stomatitis virus-based Ebola vaccine is well-tolerated and protects immunocompromised nonhuman primates. PLoS Pathog. 2008; 4(11):e1000225. [PubMed: 19043556]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Regules JA, Beigel JH, Paolino KM, et al. A recombinant vesicular stomatitis virus Ebola vaccine - preliminary report. N Engl J Med. 2015
22. Agnandji ST, Huttner A, Zinser ME, et al. Phase 1 trials of rVSV Ebola vaccine in Africa and Europe - preliminary Report. N Engl J Med. 2015
23. Mire CE, Matassov D, Geisbert JB, et al. Single-dose attenuated Vesiculovax vaccines protect primates against Ebola Makona virus. Nature. 2015; 520(7549):688–691. [PubMed: 25853476]
24. Cooper D, Wright KJ, Calderon PC, et al. Attenuation of recombinant vesicular stomatitis virus-human immunodeficiency virus type 1 vaccine vectors by gene translocations and g gene truncation reduces neurovirulence and enhances immunogenicity in mice. J Virol. 2008; 82(1): 207–219. [PubMed: 17942549]
25. Skiadopoulos MH, Surman SR, Riggs JM, et al. Evaluation of the replication and immunogenicity of recombinant human parainfluenza virus type 3 vectors expressing up to three foreign glycoproteins. Virology. 2002; 297(1):136–152. [PubMed: 12083844]
26. Durbin AP, Skiadopoulos MH, McAuliffe JM, et al. Human parainfluenza virus type 3 (PIV3) expressing the hemagglutinin protein of measles virus provides a potential method for immunization against measles virus and PIV3 in early infancy. J Virol. 2000; 74(15):6821–6831. [PubMed: 10888621]
27. Bukreyev A, Camargo E, Collins PL. Recovery of infectious respiratory syncytial virus expressing an additional, foreign gene. J Virol. 1996; 70(10):6634–6641. [PubMed: 8794298]
28. Bukreyev A, Marzi A, Feldmann F, et al. Chimeric human parainfluenza virus bearing the Ebola virus glycoprotein as the sole surface protein is immunogenic and highly protective against Ebola virus challenge. Virology. 2009; 383(2):348–361. [PubMed: 19010509]
29. DiNapoli JM, Yang L, Samal SK, et al. Respiratory tract immunization of non-human primates with a Newcastle disease virus-vectored vaccine candidate against Ebola virus elicits a neutralizing antibody response. Vaccine. 2010; 29(1):17–25. [PubMed: 21034822]
30. Yang L, Sanchez A, Ward JM, et al. A paramyxovirus-vectored intranasal vaccine against Ebola virus is immunogenic in vector-immune animals. Virology. 2008; 377(2):255–264. [PubMed: 18570964]
31. Bukreyev AA, Dinapoli JM, Yang L, et al. Mucosal parainfluenza virus-vectored vaccine against Ebola virus replicates in the respiratory tract of vector-immune monkeys and is immunogenic. Virology. 2010; 399(2):290–298. [PubMed: 20129638] ** First respirovirus-based candidate vaccine efficacious againste Ebola virus in nonhuman primates.
32. Meyer M, Garron T, Lubaki NM, et al. Aerosolized Ebola vaccine protects primates and elicits lung-resident T cell responses. J Clin Invest. 2015; 125(8):3241–3255. [PubMed: 26168222]
33. Tatsis N, Ertl HC. Adenoviruses as vaccine vectors. Mol Ther. 2004; 10(4):616–629. [PubMed: 15451446]
34. Chen H, Xiang ZQ, Li Y, et al. Adenovirus-based vaccines: comparison of vectors from three species of adenoviridae. J Virol. 2010; 84(20):10522–10532. [PubMed: 20686035]
35. Ondondo BO. The influence of delivery vectors on HIV vaccine efficacy. Front Microbiol. 2014; 5:439. [PubMed: 25202303]
36. Lasaro MO, Ertl HC. New insights on adenovirus as vaccine vectors. Mol Ther. 2009; 17(8):1333–1339. [PubMed: 19513019]
37. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008; 372(9653):1881–1893. [PubMed: 19012954]
38. Ledgerwood JE, Costner P, Desai N, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine. 2010; 29(2):304–313. [PubMed: 21034824]
39. McCoy K, Tatsis N, Korioth-Schmitz B, et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. J Virol. 2007; 81(12):6594–6604. [PubMed: 17428852]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

40. Geisbert TW, Bailey M, Hensley L, et al. Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge. J Virol. 2011; 85(9):4222–4233. [PubMed: 21325402]

41. Abbink P, Lemckert AA, Ewald BA, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. J Virol. 2007; 81(9):4654–4663. [PubMed: 17329340]

42. Xiang ZQ, Gao GP, Reyes-Sandoval A, et al. Oral vaccination of mice with adenoviral vectors is not impaired by preexisting immunity to the vaccine carrier. J Virol. 2003; 77(20):10780–10789. [PubMed: 14512528]

43. Tucker SN, Tingley DW, Scallan CD. Oral adenoviral-based vaccines: historical perspective and future opportunity. Expert Rev Vaccines. 2008; 7(1):25–31. [PubMed: 18251691]

44. Deal C, Pekosz A, Ketner G. Prospects for oral replicating adenovirus-vectored vaccines. Vaccine. 2013; 31(32):3236–3243. [PubMed: 23707160]

45. Richardson JS, Pillet S, Bello AJ, Kobinger GP. Airway delivery of an adenovirus-based Ebola virus vaccine bypasses existing immunity to homologous adenovirus in nonhuman primates. J Virol. 2013; 87(7):3668–3677. [PubMed: 23302894]

46. Vogels R, Zuijdgeest D, van Rijnsoever R, et al. Replication-deficient human adenovirus type 35 vectors for gene transfer and vaccination: efficient human cell infection and bypass of preexisting adenovirus immunity. J Virol. 2003; 77(15):8263–8271. [PubMed: 12857895]

47. Mast TC, Kierstead L, Gupta SB, et al. International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials. Vaccine. 2010; 28(4):950–957. [PubMed: 19925902]

48. Sridhar S. Clinical development of Ebola vaccines. Ther Adv Vaccines. 2015; 3(5–6):125–138. [PubMed: 26668751]

49. Sullivan NJ, Sanchez A, Rollin PE, et al. Development of a preventive vaccine for Ebola virus infection in primates. Nature. 2000; 408(6812):605–609. [PubMed: 11117750] ** First Ebola virus vaccine effective in nonhuman primates.

50. Barnes E, Folgori A, Capone S, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med. 2012; 4(115):115ra111.

51. Herath S, Le Heron A, Colloca S, et al. Analysis of T cell responses to chimpanzee adenovirus vectors encoding HIV gag-pol-nef antigen. Vaccine. 2015; 33(51):7283–7289. [PubMed: 26546736]

52. Stanley DA, Honko AN, Asiedu C, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat Med. 2014; 20(10):1126–1129. [PubMed: 25194571] * First demonstration of Ebola virus vaccine-mediated durable protection in nonhuman primates.

53. Rampling T, Ewer K, Bowyer G, et al. A monovalent chimpanzee adenovirus Ebola vaccine - preliminary report. N Engl J Med. 2015

54. Tapia MD, Sow SO, Lyke KE, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. 2016; 16(1):31–42. [PubMed: 26546548] * MVA-BN-Filo introduction into clinical trials.

55. Sullivan NJ, Hensley L, Asiedu C, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nat Med. 2011; 17(9):1128–1131. [PubMed: 21857654]

56. Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. Nat Rev Microbiol. 2009; 7(5): 393–400. [PubMed: 19369954]

57. Marzi A, Engelmann F, Feldmann F, et al. Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. Proc Natl Acad Sci U S A. 2013; 110(5):1893–1898. [PubMed: 23319647]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

58. De Santis O, Audran R, Pothin E, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. Lancet Infect Dis. 2016; 16(3):311–320. [PubMed: 26725450]
59. Rollier CS, Reyes-Sandoval A, Cottingham MG, et al. Viral vectors as vaccine platforms: deployment in sight. Curr Opin Immunol. 2011; 23(3):377–382. [PubMed: 21514130]
60. Radosevic K, Rodriguez A, Lemckert AA, et al. The Th1 immune response to Plasmodium falciparum circumsporozoite protein is boosted by adenovirus vectors 35 and 26 with a homologous insert. Clin Vaccine Immunol. 2010; 17(11):1687–1694. [PubMed: 20826614]
61. Rodriguez A, Goudsmit J, Companjen A, et al. Impact of recombinant adenovirus serotype 35 priming versus boosting of a *Plasmodium falciparum* protein: characterization of T- and B-cell responses to liver-stage antigen 1. Infect Immun. 2008; 76(4):1709–1718. [PubMed: 18212075]
62. Rodriguez A, Mintardjo R, Tax D, et al. Evaluation of a prime-boost vaccine schedule with distinct adenovirus vectors against malaria in rhesus monkeys. Vaccine. 2009; 27(44):6226–6233. [PubMed: 19686691]
63. Zahn R, Gillisen G, Roos A, et al. Ad35 and ad26 vaccine vectors induce potent and cross-reactive antibody and T-cell responses to multiple filovirus species. PLoS One. 2012; 7(12):e44115. [PubMed: 23236343]
64. Milligan ID, Gibani MM, Sewell R, et al. Safety and Immunogenicity of Novel Adenovirus Type 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. JAMA. 2016; 315(15):1610–1623. [PubMed: 27092831]
65. Zhu FC, Hou LH, Li JX, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet. 2015; 385(9984):2272–2279. [PubMed: 25817373]
66. Nwanegbo E, Vardas E, Gao W, et al. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa, and the United States. Clin Diagn Lab Immunol. 2004; 11(2):351–357. [PubMed: 15013987]
67. Croyle MA, Patel A, Tran KN, et al. Nasal delivery of an adenovirus-based vaccine bypasses pre-existing immunity to the vaccine carrier and improves the immune response in mice. PLoS One. 2008; 3(10):e3548. [PubMed: 18958172]
68. Patel A, Zhang Y, Croyle M, et al. Mucosal delivery of adenovirus-based vaccine protects against Ebola virus infection in mice. J Infect Dis. 2007; 196(Suppl 2):S413–S420. [PubMed: 17940978]
69. Choi JH, Jonsson-Schmunk K, Qiu X, et al. A single dose respiratory recombinant adenovirus-based vaccine provides long-term protection for non-human primates from lethal Ebola infection. Mol Pharm. 2015; 12(8):2712–2731. [PubMed: 25363619]
70. Peters W, Brandl JR, Lindbloom JD, et al. Oral administration of an adenovirus vector encoding both an avian influenza A hemagglutinin and a TLR3 ligand induces antigen specific granzyme B and IFN-gamma T cell responses in humans. Vaccine. 2013; 31(13):1752–1758. [PubMed: 23357198]
71. Scallan CD, Tingley DW, Lindbloom JD, et al. An adenovirus-based vaccine with a double-stranded RNA adjuvant protects mice and ferrets against H5N1 avian influenza in oral delivery models. Clin Vaccine Immunol. 2013; 20(1):85–94. [PubMed: 23155123]
72. Gilbert SC. Clinical development of Modified Vaccinia virus Ankara vaccines. Vaccine. 2013; 31(39):4241–4246. [PubMed: 23523410]
73. O'Hara GA, Duncan CJ, Ewer KJ, et al. Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. J Infect Dis. 2012; 205(5):772–781. [PubMed: 22275401]
74. Berthoud TK, Hamill M, Lillie PJ, et al. Potent $CD8^+$ T-cell immunogenicity in humans of a novel heterosubtypic influenza A vaccine, MVA-NP+M1. Clin Infect Dis. 2011; 52(1):1–7. [PubMed: 21148512]
75. Cottingham MG, Carroll MW. Recombinant MVA vaccines: dispelling the myths. Vaccine. 2013; 31(39):4247–4251. [PubMed: 23523407]
76. Wolferstatter M, Schweneker M, Spath M, et al. Recombinant modified vaccinia virus Ankara generating excess early double-stranded RNA transiently activates protein kinase R and triggers enhanced innate immune responses. J Virol. 2014; 88(24):14396–14411. [PubMed: 25297997]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

77. Martin JE, Sullivan NJ, Enama ME, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol. 2006; 13(11):1267–1277. [PubMed: 16988008]

78. Sarwar UN, Costner P, Enama ME, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. J Infect Dis. 2015; 211(4):549–557. [PubMed: 25225676]

79. Kibuuka H, Berkowitz NM, Millard M, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. Lancet. 2015; 385(9977):1545–1554. [PubMed: 25540891]

80. Amante DH, Smith TR, Mendoza JM, et al. Skin transfection patterns and expression kinetics of electroporation-enhanced plasmid delivery using the CELLECTRA-3P, a portable next-generation dermal electroporation device. Hum Gene Ther Methods. 2015; 26(4):134–146. [PubMed: 26222896]

81. Wang Y, Liu Z, Dai Q. A highly immunogenic fragment derived from Zaire Ebola virus glycoprotein elicits effective neutralizing antibody. Virus Res. 2014; 189:254–261. [PubMed: 24930448]

82. Swenson DL, Warfield KL, Negley DL, et al. Virus-like particles exhibit potential as a pan-filovirus vaccine for both Ebola and Marburg viral infections. Vaccine. 2005; 23(23):3033–3042. [PubMed: 15811650]

83. Lovgren Bengtsson K, Morein B, Osterhaus AD. ISCOM technology-based Matrix M adjuvant: success in future vaccines relies on formulation. Expert Rev Vaccines. 2011; 10(4):401–403. [PubMed: 21506635]

84. Smith G, Raghunandan R, Wu Y, et al. Respiratory syncytial virus fusion glycoprotein expressed in insect cells form protein nanoparticles that induce protective immunity in cotton rats. PLoS One. 2012; 7(11):e50852. [PubMed: 23226404]

85. Bosio CM, Moore BD, Warfield KL, et al. Ebola and Marburg virus-like particles activate human myeloid dendritic cells. Virology. 2004; 326(2):280–287. [PubMed: 15302213]

86. Swenson DL, Warfield KL, Kuehl K, et al. Generation of Marburg virus-like particles by co-expression of glycoprotein and matrix protein. FEMS Immunol Med Microbiol. 2004; 40(1):27–31. [PubMed: 14734183]

87. Warfield KL, Bosio CM, Welcher BC, et al. Ebola virus-like particles protect from lethal Ebola virus infection. Proc Natl Acad Sci U S A. 2003; 100(26):15889–15894. [PubMed: 14673108]

88. Warfield KL, Olinger G, Deal EM, et al. Induction of humoral and CD8+ T cell responses are required for protection against lethal Ebola virus infection. J Immunol. 2005; 175(2):1184–1191. [PubMed: 16002721]

89. Warfield KL, Posten NA, Swenson DL, et al. Filovirus-like particles produced in insect cells: immunogenicity and protection in rodents. J Infect Dis. 2007; 196(Suppl 2):S421–S429. [PubMed: 17940979]

90. Warfield KL, Swenson DL, Demmin G, Bavari S. Filovirus-like particles as vaccines and discovery tools. Expert Rev Vaccines. 2005; 4(3):429–440. [PubMed: 16026254]

91. Martins KA, Steffens JT, van Tongeren SA, et al. Toll-like receptor agonist augments virus-like particle-mediated protection from Ebola virus with transient immune activation. PLoS One. 2014; 9(2):e89735. [PubMed: 24586996]

92. Martins KA, Warren TK, Bavari S. Characterization of a putative filovirus vaccine: virus-like particles. Virol Sin. 2013; 28(2):65–70. [PubMed: 23385315]

93. Martins K, Carra JH, Cooper CL, et al. Cross-protection conferred by filovirus virus-like particles containing trimeric hybrid glycoprotein. Viral Immunol. 2015; 28(1):62–70. [PubMed: 25514232]

94. Martins KA, Cooper CL, Stronsky SM, et al. Adjuvant-enhanced CD4 T cell responses are critical to durable vaccine immunity. EBioMedicine. 2016; 3:67–78. [PubMed: 26870818]

95. Swenson DL, Warfield KL, Larsen T, et al. Monovalent virus-like particle vaccine protects guinea pigs and nonhuman primates against infection with multiple Marburg viruses. Expert Rev Vaccines. 2008; 7(4):417–429. [PubMed: 18444889]

96. Warfield KL, Swenson DL, Negley DL, et al. Marburg virus-like particles protect guinea pigs from lethal Marburg virus infection. Vaccine. 2004; 22(25–26):3495–3502. [PubMed: 15308377]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

97. Warfield KL, Swenson DL, Olinger GG, et al. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. J Infect Dis. 2007; 196(Suppl 2):S430–S437. [PubMed: 17940980] ** First study demonstrating efficacy of a virion-like particle vaccine in nonhuman primates.

98. Kilgore N, Nuzum EO. An interagency collaboration to facilitate development of filovirus medical countermeasures. Viruses. 2012; 4(10):2312–2316. [PubMed: 23202465]

99. Bradfute SB. Duration of immune responses after Ebola virus vaccination. Lancet Infect Dis. 2016; 16(1):2–3. [PubMed: 26546547]

100. Patel M, Shane AL, Parashar UD, et al. Oral rotavirus vaccines: how well will they work where they are needed most? J Infect Dis. 2009; 200(Suppl 1):S39–S48. [PubMed: 19817613]

101. Qadri F, Bhuiyan TR, Sack DA, Svennerholm AM. Immune responses and protection in children in developing countries induced by oral vaccines. Vaccine. 2013; 31(3):452–460. [PubMed: 23153448]

102. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Biol. 2010; 8:129. [PubMed: 20920375]

103. Qiu X, Fernando L, Alimonti JB, et al. Mucosal immunization of cynomolgus macaques with the VSVDeltaG/ZEBOVGP vaccine stimulates strong ebola GP-specific immune responses. PLoS One. 2009; 4(5):e5547. [PubMed: 19440245]

104. Geisbert TW, Daddario-Dicaprio KM, Geisbert JB, et al. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. Vaccine. 2008; 26(52):6894–6900. [PubMed: 18930776]

105. Pratt WD, Wang D, Nichols DK, et al. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. Clin Vaccine Immunol. 2010; 17(4):572–581. [PubMed: 20181765]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

KEY ISSUES

- Multiple vaccines targeting Ebola virus are currently in clinical trials
- Vectored vaccines, including vaccines in adenovirus, vesicular stomatitis Indiana virus, and poxvirus vectors, are the most advanced in clinical trials
- Ebola virus-specific vaccines vary in their safety and immunogenicity profiles
- Vaccines to prevent Ebola virus disease should be developed with consideration for the region in which the pathogen is emerging
- Vaccines that are efficacious after a single vaccination, have a strong safety profile, do not require cold-chain storage, and induce mucosal immunity may be ideal for protection against filovirus infection and/or disease
- The rVSV-ZEBOV vaccine is the only vaccine for which human efficacy data exist

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Overview of Ebola virus disease candidate vaccines under clinical investigation.

Vaccine Class	Vaccine Name	Manufacturer or sponsor	Most Advanced Clinical Trial Status*	Pros	Cons	Comments
Vectored, Live	rVSV-ZEBOV [12–14,17–19,21,22]	Merck Sharp & Dohme Corp, NewLink Genetics, Public Health Agency of Canada	Phase 3	Single vaccination is highly immunogenic	Some safety concerns	
	HPIV3-EbovZ GP [30–32]	NIAID	Phase 1	Intranasal administration may elicit more robust mucosal immunity	Pre-existing immunity to vector	
Vectored, non-replicating	ChAd3.EBOZ / ChAd3.EBO [52–57]	Glaxo Smith Kline, NIAID	Phase 2	Safety	Requires boost vaccination	
	Ad26.ZEBOV [40,63]	Crucell Holland BV (Johnson & Johnson)	Phase 3	Safety	Requires boost vaccination	Phase 4 retrospective evaluation underway though product is not licensed (NCT02661464)
	Ad5-EBOV [65,66]	Tianjin CanSino Biotechnology and Beijing Institute of Biotechnology	Phase 2	Pre-existing immunity to Ad5 vector	Requires boost vaccination	
	VRC-EBOADV018-00-VP [38,39]	NIAID	Phase 1	Pre-existing immunity to Ad5 vector	Requires boost vaccination	No longer active
	MVA-BN-Filo [54]	Bavarian Nordic GmbH / Crucell Holland BV (Johnson & Johnson)	Phase 3	Safety	Immunogenicity; Requires heterotypic pairing for vaccine administration	Phase 4 retrospective evaluation underway though product is not licensed (NCT02661464)
	MVA-EbolaZ (VRC-EBOMVA079-00-VP)[52]	NIAID	Phase 1	Safety	Immunogenicity; Requires heterotypic pairing for vaccine administration	
DNA	VRC-EBODNA023-00-VP, VRCEBODNA012-00-VP, and VRCMARDNA025-00-VP [49,77–79]	NIAID	Phase 1	Safety, flexible platform, low cost	Effective administration requires electroporation technology; requires boost vaccination for immunogenicity	No longer active

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Vaccine Class	Vaccine Name	Manufacturer or sponsor	Most Advanced Clinical Trial Status*	Pros	Cons	Comments
	INO-4201, -4202, and -4212 [no publications yet]	Inovio Pharmaceuticals, GeneOne Life Science Inc., DARPA	Phase 1	Safety, flexible platform, low cost	Effective administration requires electroporation technology; requires boost vaccination for immunogenicity	
Subunit / Protein	EBOVGP$_{1,2}$ with Matrix-M [no publications yet])	Novavax	Phase 1	Pending Phase 1 results, "pro" may be safety	Immunogenicity unclear at time of publication	

* According to https://clinicaltrials.gov.