Lessons learned from Zaire ebolavirus to help address urgent needs for vaccines against Sudan ebolavirus and Marburg virus

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ABSTRACT

The 2014–2016 Ebola virus epidemic in West Africa triggered extensive investments from public and private partners in an attempt to slow the spread of disease and bring the outbreak under control. This significantly accelerated the pace of development of countermeasures against Zaire ebolavirus that enabled vaccines to be a part of an effective response to the most recent 2018–2019 outbreak in the Democratic Republic of the Congo. However, there remain urgent and unmet needs for medical countermeasures against other members of the Filoviridae family that cause viral hemorrhagic fevers. To improve the national and global preparedness posture for viral hemorrhagic fevers, a renewed emphasis is being placed on developing vaccines for filoviruses other than Zaire ebolavirus. Here we discuss lessons learned from the West Africa epidemic and how those lessons apply to the development of vaccine candidates for other filoviruses, specifically Sudan ebolavirus and Marburg virus. This commentary will highlight some of the key product development gaps to address in preparation for future disease outbreaks caused by these viruses.

Introduction

The Biomedical Advanced Research and Development Authority (BARDA), part of the U.S. Department of Health and Human Services’ (HHS) Office of the Assistant Secretary of Preparedness and Response (ASPR), supports innovation, development, and procurement of Medical Countermeasures (MCM), including vaccines, drugs, and diagnostics, against a broad array of public health threats. Among biological threats, the filoviruses (Ebola virus and Marburg virus) represent priority pathogens for vaccine development. These viruses represent potential threats through bioterrorism, as well as naturally occurring public health threats as highlighted by the 2014 to 2016 epidemic in West Africa and the current outbreak ongoing in the Democratic Republic of the Congo (DRC), which was declared by the World Health Organization in August 2018.

Since 2014, vaccines targeting Zaire ebolavirus (EBOV), the species that caused both the West Africa epidemic and the ongoing outbreak, have progressed rapidly. The epidemic galvanized investments from public and private partnerships enabling rapid progress from preclinical development in mid-2014 to a vaccine licensed by the European Medicines Agency (EMA) in 2019. The rVSV-ZEBOV-GP vaccine, now referred to as ERVEBO®, was deployed prior to its licensure as part of a broader strategy to control the 2018–2019 outbreak in the Democratic Republic of the Congo. In this outbreak, public health measures were implemented in a similar manner to previous outbreaks, including contact tracing, emphasis on hygiene, and safe burial practices, but the vaccine represented the introduction of a new tool to help contain the outbreak. Together with other partners, BARDA invested in candidate EBOV vaccines to advance the development of these vaccines and improve the nation’s and the world’s collective preparedness posture in the event of future EBOV outbreaks. As of mid-January, 2020 over 262,000 individuals had been vaccinated with ERBEVO® in the DRC alone. In parallel, a second vaccine using an Adenovirus serotype 26 prime and a modified Vaccinia Ankara boost to deliver EBOV antigens is now also being evaluated in the DRC and Rwanda.

Emerging infectious diseases are a constant, but evolving, threat to public health. Zika virus emerged as a virus of concern, mainly due to its relationship with clusters of microcephaly and Guillain-Barre syndrome, in 2015. New strains of influenza continue to emerge and threaten with pandemic potential. In early 2020, a novel coronavirus has emerged and caused thousands of human infections in January alone. The history of filovirus outbreaks has included several sporadic events caused by EBOV, Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV), and Marburg virus (MARV), along with isolated cases of Tai Forest ebolavirus (TAFV) and serologic evidence of Reston ebolavirus (RESTV) infections. A sixth species of Ebola virus, Bombali ebolavirus, was only recently discovered, further highlighting the evolving nature of the potential threat posed by filoviruses. As recently as October 2017, Uganda experienced a small outbreak of disease caused by MARV. This outbreak was quickly contained by an effective public health response, while also highlighting the need for countermeasures against a broader array of filoviruses to combat future outbreaks.
EBOV vaccine product development gaps – lessons learned

In early 2014, a major gap in EBOV vaccine preparedness existed as critical clinical data was unavailable to support the use of EBOV vaccines in the field. EBOV vaccines with clinical data were limited to Adenovirus serotype 5\(^{11}\) and DNA-based constructs,\(^{12}\) and the clinical development of these programs ended after Phase 1 studies. Multiple potential vaccine candidates had shown proof-of-concept efficacy in animal models including viral replicons,\(^{13}\) virus-like particles,\(^{14}\) vesicular stomatitis virus (VSV) vectors\(^{15}\) and next-generation Adenovirus vectors.\(^{16,17}\) The development of EBOV vaccines progressed rapidly during the epidemic in West Africa, with clinical trials eventually being initiated in Liberia, Guinea, and Sierra Leone in February, March, and April 2015, respectively. However, there were a number of priority gaps that had to be addressed prior to the use of any of the EBOV vaccines in the field. Table 1 provides a summary of the product development gaps for EBOV vaccines as of early 2014.

Clinical development

Assessments of the human safety and immunogenicity of EBOV vaccine candidates were not available at the beginning of the 2014 epidemic, highlighting significant development gaps of these vaccines at that time. Before the Phase 2 and 3 trials could be conducted in Liberia, Guinea, and Sierra Leone, a number of Phase 1 trials were required to ensure clinical safety of lead vaccine candidates in late 2014.\(^{9}\) The safety and immunogenicity data arising from those trials ultimately informed the choice of vaccine candidates that would be assessed in West Africa. However, dose ranging studies had not been completed, so several of the early trials evaluated a range of doses and schedules that also informed the clinical doses used in Phase 2 and 3 clinical trials.

Nonclinical development

Prior to clinical development, selection of candidate vaccines in pre-clinical stages were largely guided by efficacy in animal models; it was important that the animal model was consistent among studies in terms of the animal species, virus strain, and route of exposure. To address this need, most nonhuman primate challenge studies used cynomolgus macaques as the host species. This species is the current standard for evaluation of vaccine-mediated protection against viral hemorrhagic fever caused by filoviruses.\(^{18}\) The Filovirus Animal and Non-Clinical Working Group (FANG) had identified potential strains of EBOV that may meet the guidelines set forth in the FDA Animal Rule guidance.\(^{19}\) Recent work highlighted the importance of maintaining minimally passaged isolates of these viruses to avoid attenuating mutations and phenotypical changes to the viral particle-plaque forming unit (pfu) ratio.\(^{20,21}\) The need for a nonhuman primate challenge model, combined with the complexities of conducting studies under high containment (biosafety level 4), presented significant challenges in conducting statistically powered studies to evaluate the effects of dosing on immunogenicity and survival. In addition, while proof-of-concept data were available for a number of vaccine candidates,\(^ {22}\) studies in well-characterized animal models with vaccines produced under current Good Manufacturing Practices (cGMP) conditions had not been completed.

Nonclinical safety and toxicology assessments were vital to the rapid progress of EBOV vaccines, but many lead candidates had not yet undergone nonclinical toxicology studies under Good Laboratory Practices (GLP) regulations. For example, multiple non-GLP nonclinical studies had been published for the rVSV\(\Delta\)G-EBOV vaccine suggesting that it was safe in mice, guinea pigs, and nonhuman primates.\(^{23,24}\) Additional GLP studies had to be performed in accordance with well-defined protocols to generate full toxicology reports. Considerations for GLP toxicology studies were reviewed by Al-Humadi, et al.\(^ {25}\) and these GLP studies are generally expected to support Investigational New Drug Applications prior to clinical development. Such studies enable an independent assessment of the safety of vaccine prior to clinical development.

As the Phase 1 clinical trials and supportive animal efficacy studies progressed, the need for immunological assays to assess immune responses in humans and animals became an issue. Without immunological assays, there would have been no way to assess the likelihood of protection in humans. A number of these assays were developed, such as glycoprotein ELISAs to measure total antibody levels, neutralization assays, and various cellular immune response assays (IFN-\(\gamma\)

Table 1. Key gaps in Ebola vaccine development as of early 2014.

| Category                        | Gap                                      | Status as of Early 2014                                      |
|---------------------------------|------------------------------------------|-------------------------------------------------------------|
| Clinical                        | Safety and Immunogenicity Data           | Phase 1 data from Ad5- and DNA-vectorized vaccines only.   |
|                                 | Dose Selection                           | No dose selection to move into further development.         |
|                                 | Immune Assays                            | ELISA, intracellular T cell staining, and ELISPOT used to assess immunology. |
| Non-Clinical                    | Efficacy in Nonhuman Primates            | Proof of concept efficacy available for VRP, VLP, Adenovirus, VSV, DNA. |
|                                 | GLP Toxicology                           | GLP toxicology lacking for these leads.                     |
|                                 | Concurrence on Animal Species, Route of Challenge, and Challenge Strains | No FDA concurrence on animal species, route of challenge, or challenge strains. |
|                                 | Lot Release Assays                       | Limited number of assays available.                         |
| Chemistry, Manufacturing, and Controls | Small Scale Process Development         | Processes available for rVSV\(\Delta\)G-EBOV; Lab scale processes developed for other leads. |
|                                 | cGMP Manufactured Lots                   | cGMP lot of rVSV\(\Delta\)G-EBOV; No other cGMP lots ready for clinical evaluation. |
|                                 | Stability Data to Support Clinical Studies | Limited understanding of stability profiles.                 |

\*VRP = Viral Replicon, VLP = Virus-Like Particle, VSV = Vesicular Stomatitis Virus, GLP = Good Laboratory Practices, cGMP = current Good Manufacturing Practices.
ELISpot and intracellular cytokine staining). Emphasis was placed on antibody-based assays as ELISA titers appeared to correlate with protection in animal models. An anti-EBOV glycoprotein IgG ELISA was eventually qualified and validated to assess the immune response in both nonhuman primates and human clinical samples. These efforts highlighted that both the development of the assays themselves and the maintenance of critical reagents such as recombinant glycoprotein and reference sera are of vital importance to the immunological analyses of nonclinical and clinical samples.

Chemistry, Manufacturing, and Controls (CMC)

Prior to entering clinical development, it was critical that vaccine candidates be produced under current Good Manufacturing Practices (cGMP). Availability of cGMP manufactured vaccine lots, along with appropriate tests and assays for product release and ongoing stability testing, was a key gap for many lead EBOV vaccine candidates as of early 2014, as was an inventory of cGMP material. Thus, funding and resources to further develop and optimize the manufacturing processes and manufacture cGMP grade material were key requisites to enable submission of Investigational New Drug (IND) applications to the FDA.

For any vaccine candidate, lot release assays are essential to demonstrate that each given lot meets standards for potency, identity, purity, and sterility. Standardized assays are available to measure key parameters such as pH, endotoxin, and host cell DNA. However, product-specific assays are needed to measure the potency and identity. In the case of the EBOV vaccines, these non-compendial assays, which may be specific to the vaccine in question, required further development for most candidates in 2014. For example, in vitro infectivity assays were needed as a demonstration of potency, and Western blots had to be developed to prove identity.

Stability is also a key piece of the overall CMC development. As EBOV candidates progressed toward clinical development, demonstration of stability became an important component of the program. For any vaccine lot to be considered for use in clinical studies, supportive data showing that the lot will remain stable for the duration of those studies are necessary. Stability profiles were not well understood for lead candidates at the outset of the epidemic. Therefore, stability data had to be generated in real time, under multiple storage conditions, to inform the eventual storage and handling conditions.

Application of lessons learned to Sudan ebolavirus and Marburg virus

Clinical development

While we now have a licensed vaccine against EBOV, those targeting SUDV and MARV are in early stages of development. The current state of preparedness for SUDV and MARV outbreaks, in terms of being able to incorporate vaccine(s) into the overall public health response, is similar to the landscape for EBOV in early 2014 but is now improving. SUDV and MARV constructs were included in Phase 1 clinical trials conducted prior to the West Africa epidemic, using Adenovirus serotype 5 and DNA-based vaccines. During the West Africa epidemic, some of the Phase 1 trials with the ChAd3 vector included a bivalent EBOV/SUDV mixture. Phase 1 trials are now underway for monovalent constructs of the ChAd3 vector expressing the MARV GP and the SUDV GP (from https://clinicaltrials.gov/, NCT03475056 and NCT04041570). As of the end of 2019, the pipeline for clinical development of SUDV and MARV vaccines is starting to grow but additional candidates in clinical stage development may be needed.

Nonclinical development

There are multiple vaccine candidates with promising efficacy data in nonhuman primate models of infection. Reynolds, et al., and Suschak, et al., both recently reviewed vaccine approaches for EBOV, SUDV, and MARV, highlighting candidates that have been assessed in nonhuman primate models of infection. Only virus-like particles, viral replicons, adenovirus vectors, and vesicular stomatitis virus vectors have published efficacy results for challenges with SUDV; in addition to those platforms, inactivated virus and DNA constructs have also been assessed for nonhuman primate efficacy against MARV.

Given the sporadic nature of SUDV and MARV outbreaks, licensure of vaccines will likely occur via the FDA Animal Rule. Building on lessons learned from the EBOV response efforts, the filovirus research community has come to a general consensus regarding strains to be used for SUDV (Gulu strain) and MARV (Angola strain) studies, suggesting that only passage two or three material should be utilized. Multiple groups have recently investigated alternative animal models such as marmosets and ferrets, as well as different routes of mucosal challenge to more closely replicate the types of exposure that may occur in a clinical setting. However, intramuscular challenges in cynomolgus macaques, generally with a targeted 1,000 pfu, remain the standard challenge against which vaccine efficacy is measured. Assuming licensure of MARV and SUDV vaccines will occur via the Animal Rule, concurrence on the animal models from regulatory authorities will be essential. BARDA will be supporting natural history studies for both SUDV and MARV with that goal in mind.

While a variety of different candidates have demonstrated promising results in non-clinical efficacy studies, there must be an emphasis on GLP toxicology studies and assay development. It is essential that assays to quantify immune responses to SUDV and MARV vaccines, and the animal models in which these vaccines will be assessed, remain a focus for the filovirus research community. While antibodies binding to GP were measured by a validated ELISA for EBOV, independent efforts may be needed for each individual MARV and SUDV candidate to determine what the immune correlate(s) may be and develop the relevant assay(s). A variety of different assays are in development to assess neutralizing antibody titers, which may provide a more functional readout of the immune response. Key immunological assays that correlate with protection in nonclinical challenge models will require qualification and validation.
CMC

Similar to EBOV in early 2014, vaccine candidates for SUDV and MARV will need substantial investments in the CMC development space. This will be driven largely by the need for cGMP vaccine lots and the potential scale of manufacturing. Compendial methods, such as measuring pH, osmolality, sterility, bioburden, and endotoxin, are relatively straightforward to apply. However, non-compendial methods will require additional development, qualification, and validation. These assays will be product-specific and will include additional assays to measure purity as well as potency and identity. For example, potency assays may include in vitro infectivity assays for viral-vector vaccines but could require in vivo immunogenicity and/or efficacy for candidates that may not be amenable to in vitro assays, such as recombinant proteins or other non-replicating platforms. Identity assays may include ELISAs, Western blots, and PCR, among others, and will also be specific to the individual candidate and the specific antigen used as the vaccine.

Scale of production will also be a key consideration in CMC development for lead candidates and is much more difficult to address. The largest SUDV and MARV outbreaks have consisted of hundreds of cases. However, the same was true of EBOV prior to the 2014 West Africa epidemic. As a frame of reference, more than 250,000 vaccine doses were administered as of the end of 2019 as part of the ongoing response in the Democratic Republic of the Congo, which has resulted in just over 3,300 cases and 2,200 deaths as of December 2019. The Global Alliance for Vaccines Initiative recently announced an agreement to establish and maintain a stockpile of 500,000 vaccine doses. Continued stakeholder engagement among parties that are likely to need supplies of these vaccines will be vital to ensuring an appropriate commercial scale for SUDV and MARV vaccines and long-term planning that will balance the need for vaccines while maintaining sustainability in terms of manufacturing capacity and costs. Regardless, some level of manufacturing scale up would be expected for any vaccine candidate at this stage. Even at early stages of development, consideration should be given to the manufacturing processes that are needed for the vaccine, and if any major technical hurdles may limit the scalability. The summary of product development gaps as of December 2019 for SUDV and MARV vaccines is provided in Table 2.

Summary

Additional investments in vaccines against SUDV and MARV are needed to continue to advance candidates to the stage at which they could be considered for use in response to an outbreak. In short, an ideal situation would include having clinical safety and immunogenicity data for multiple lead candidates. This would be supplemented by nonclinical toxicity and efficacy data in relevant animal models. However, the clinical and nonclinical data will be of limited use if an inventory of cGMP grade clinical trial material is unavailable. That said, multiple EBOV vaccine candidates have progressed into clinical development. The data generated and lessons learned from those clinical trials may be informative in selecting vaccine vectors that could be applied to SUDV and MARV.

The near-term objectives of the BARDA Medical Countermeasures Vaccine Program include supporting critical CMC activities, such as assay development, process development, cGMP manufacturing runs, and stability assessments to ensure the availability of downstream supply of cGMP-grade material to respond to an outbreak. In parallel, clinical and nonclinical objectives include assessments of lead candidates through Phase 2 clinical trials and nonclinical efficacy studies, pushing toward an intermediate state of development to enable Emergency Use Authorization under FDA guidelines (https://www.fda.gov/RegulatoryInformation/Guidances/ucm125127.htm#euas) while continuing to advance toward licensure. With a licensed EBOV vaccine paving the way, BARDA’s goal is to have licensed monovalent vaccines for SUDV and MARV within the next decade, dramatically improving the nation’s and the world’s collective preparedness posture for filovirus outbreaks.

Table 2. Current State of SUDV and MARV gaps as of the end of 2019.

| Category                                      | Gap                                                                 | Current Status SUDV                                                                 | Current Status MARV                                                                 |
|-----------------------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Clinical Safety and Immunogenicity Data       | Phase 1 data from ChAd3, Ad5, DNA constructs. Phase 1 underway for multivalent Ad26/MVA. | Phase 1 data from DNA construct. Phase 1 underway for ChAd3 and multivalent Ad26/ MVA. |                                                                                   |
| Clinical Dose Selection                       | No dose selection for further studies. Qualified immune assays needed. Proof of concept available for VSV, VRP, VLP, Ad5, DNA, ChAd3. | No dose selection for further studies. Qualified immune assays needed. Proof of concept available for VSV, VLP, DNA, Ad5, ChAd3. |                                                                                   |
| Clinical Immune Assays                        | GLP Toxicology GLP toxicity for ChAd3 and multivalent Ad26/ MVA. GMP lots available for ChAd3 and Ad26/MVA. | GLP toxicity for ChAd3 and multivalent Ad26/MVA. GMP lots available for ChAd3 and Ad26/MVA. |                                                                                   |
| Clinical Efficacy in Nonhuman Primates        | Concurrence on Animal Species, Route of Challenge, and Challenge Strains Lot Release Assays Development, qualification, and validation required for key non-compendial assays. Lab-scale development for ChAd3, DNA, and Ad26/MVA vectors. | No official concurrence on animal species, virus strain, or route of challenge. Development, qualification, and validation required for key non-compendial assays. Lab-scale development for ChAd3, DNA, and Ad26/MVA vectors. |                                                                                   |
| Non-Clinical GLP Toxicology                  | GLP Toxicology GLP toxicity for ChAd3 and multivalent Ad26/ MVA. GMP lots available for ChAd3 and Ad26/MVA. | GLP toxicity for ChAd3 and multivalent Ad26/MVA. GMP lots available for ChAd3 and Ad26/MVA. |                                                                                   |
| Non-Clinical Concurrence on Animal Species, Route of Challenge, and Challenge Strains Lot Release Assays Development, qualification, and validation required for key non-compendial assays. Lab-scale development for ChAd3, DNA, and Ad26/MVA vectors. | No official concurrence on animal species, virus strain, or route of challenge. Development, qualification, and validation required for key non-compendial assays. Lab-scale development for ChAd3, DNA, and Ad26/MVA vectors. |                                                                                   |
| Non-Clinical GLP Toxicology                  | GLP Toxicology GLP toxicity for ChAd3 and multivalent Ad26/ MVA. GMP lots available for ChAd3 and Ad26/MVA. | GLP toxicity for ChAd3 and multivalent Ad26/MVA. GMP lots available for ChAd3 and Ad26/MVA. |                                                                                   |
| Non-Clinical Concurrence on Animal Species, Route of Challenge, and Challenge Strains Lot Release Assays Development, qualification, and validation required for key non-compendial assays. Lab-scale development for ChAd3, DNA, and Ad26/MVA vectors. | No official concurrence on animal species, virus strain, or route of challenge. Development, qualification, and validation required for key non-compendial assays. Lab-scale development for ChAd3, DNA, and Ad26/MVA vectors. |                                                                                   |

*VRP = Viral Replicon, VLP = Virus-Like Particle, VSV = Vesicular Stomatitis Virus, GLP = Good Laboratory Practices, cGMP = current Good Manufacturing Practices, Ad5 = Adenovirus serotype 5, Ad26 = Adenovirus serotype 26, ChAd3 = Chimpanzee Adenovirus serotype 3, MVA = Modified Vaccinia Ankara.
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References

1. Agency EM Ervebo; 2019 Dec 17 [accessed 2019 Dec 20]. https://www.ema.europa.eu/en/medicines/human/EPAR/erievebo.
2. Administration FD First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response. 2019 [accessed 2019 Dec 17]. https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health
3. Wolfe DN, Zarrabian AG, Disbrow GL, Espeland EM. Progress towards a vaccine against Ebola to meet emergency medical countermeasure needs. Vaccine. 2017;17:7178–82.
4. Heymann D, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, Baruah K, Mahmud G, Kandun N, Vasconcelos PFC, et al. Zika virus and microcephaly: why is this situation a PHEIC. Lancet. 2016;387(10020):719–21. doi:10.1016/S0140-6736(16)00320-2.
5. Taubengerger J, Morens DM. Pandemic influenza - including pandemic influenza - milestone-public-health
6. Silva W, Das TK, Izuireta R. Estimating disease burden of hemorrhagic fever. J Infect Dis. 2009;200. Dec 17 [accessed 2019 Dec 20].
7. Geisbert T, Strong JE, Feldmann H. Considerations in the use of nonhuman primate models of Ebola virus and Marburg virus infection. J Infect Dis. 2015;212(Supplement 2):S91–97. doi:10.1093/infdis/jiv284.
8. Cross R, Mire CE, Feldmann H, Geisbert TW. Post-exposure treatments for Ebola and Marburg virus infections. Nat Rev Drug Discov. 2018;17(22):1812–24. doi:10.1038/s41573-018-00476-y.
9. Administration, F.a.D. Product development under the animal rule. Guidance for Industry. C.F.D.E.A.R.C.F.B.E.a. Research, Editor. 2015 [accessed 2019 Dec 12]. https://www.fda.gov/downloads/drugs/guidances/ucm399217.pdf
10. Goldstein T, Anthony SJ, Gbakima A, Bird BH, Bangura J, et al. Particle-to-FPU ratio of Ebola virus influences disease course and survival in cynomolgus macaques. J Virol. 2015;89(13):6773–81. doi:10.1128/JVI.00649-15.
11. Ledgerwood J, Costner P, Desai N, Holman L, Enama ME, Johnson JC, Hensley L, Ammendola V, Abbate A, Grazioli F, et al. Chimpanzee adenosine virus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat Med. 2014;20(10):1126–29. doi:10.1038/nm.3702.
12. Lawrence P, Guignard J, Johnson JC, Kandun N, Vasconcelos PFC, et al. Ebola virus vectored vaccine protects rhesus monkeys from intramuscular and aerosol challenge with ebolavirus. J Virol. 2013;87(9):4952–64. doi:10.1128/JVI.03361-12.
29. Reynolds P, Marzi A. Ebola and Marburg virus vaccines. Virus Genes. 2017;53(4):501–15. doi:10.1007/s11262-017-1455-x.

30. Suschak J, Schmaljohn CS. Vaccines against Ebola virus and Marburg virus: recent advances and promising candidates. Hum Vaccines Immunotherapeutics. 2019;15(10):2359–77. doi:10.1080/21645515.2019.1651140.

31. Hirschberg R, Ward LA, Kilgore N, Kurnat R, Schiltz H, Albrecht MT, Christopher GW, Nuzum E. Challenges, progress, and opportunities: proceedings of the Filovirus medical countermeasures workshop. Viruses. 2014;6(7):2673–97. doi:10.3390/v6072673.

32. Smither SJ, Nelson M, Eastaugh L, Nunez A, Salguero FJ, Lever MS. Experimental respiratory infection of marmosets (Callithrix jacchus) with Ebola virus kikwit. J Infect Dis. 2015;212(Suppl 2):S336–345. doi:10.1093/infdis/jiv371.

33. Kroeker A, He S, de La Vega M, Wong G, Embury-Hyatt C, Qiu X. Characterization of Sudan Ebolavirus infection in ferrets. Oncotarget. 2017;8(28):46262–72. doi:10.18632/oncotarget.17694.

34. Cross RW, Mire CE, Borsevich V, Geisbert JB, Fenton KA, Geisbert TW. The domestic ferret (Mustela putorius furo) as a lethal infection model for 3 species of ebolavirus. J Infect Dis. 2016;214(4):565–69. doi:10.1093/infdis/jiw209.

35. Alston K, Avena LE, Woowa G, Carrion R, Griffiths A. Development of a lethal intranasal exposure model of Ebola virus in the cynomolgus macaque. Viruses. 2017;9(11):319. doi:10.3390/v9110319.

36. Organization WH, Ebola Virus Disease; Democratic Republic of the Congo External Situation Report 71. 2019: https://www.who.int/emergencies/diseases/ebola/drc-2019/situation-reports.

37. Alliance GTV Gavi Board approves new Ebola vaccine programme. 2019 [accessed 2019 Dec 6]. https://www.gavi.org/news/media-room/gavi-board-approves-new-ebola-vaccine-programme