

EDITORIAL



Ebola virus – prospects for a novel virus-like-particle-expressing modified vaccinia Ankara-based vaccine

Harriet L. Robinson ^a and Andrea Marzi ^b

^aGeoVax Inc., Atlanta, GA, USA; ^bLaboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

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The Ebola virus (EBOV) epidemic in West Africa that devastated Guinea, Liberia, and Sierra Leone from 2013 to 2016 became a global public health crisis and claimed over 11,000 human lives [1]. There were no licensed vaccines and therapeutics against EBOV disease available at the time of the epidemic, but several experimental vaccine approaches were accelerated into human clinical trials starting in October 2014 [2]. Among these a replication competent vesicular stomatitis virus (VSV) vector expressing the EBOV glycoprotein (GP) in place of the VSV glycoprotein (VSV-EBOV, also known as rVSV-ZEBOV) met with single-dose success, a success that was demonstrated using a ring vaccination strategy [3]. This vaccine is moving toward licensure in the United States and Europe, but has already been licensed as a combination vaccine in Russia [4]. However, VSV-EBOV has disadvantages. As it was developed as an emergency vaccine, it is fast-acting due to its replication competence in the vaccinated individual, but can cause mild adverse events similar to those observed by Yellow fever vaccination, for example, pain at injection site and fever [5]. In addition, a single study showed that the VSV-EBOV vaccine requires long-term storage conditions at -70°C and has limited stability at 4°C and 25°C [6], although the manufacturer's are currently working toward a lyophilized formulation. If multiple doses of the VSV-EBOV have to be administered to achieve complete protection its use would be limited as the vaccine doses require a stable cold-chain for long-term storage which is challenging in Africa; however, as a single-dose vaccine, its stability is appropriate for where it is most needed in the developing world during outbreaks. However, several other experimental vaccine approaches have been developed to support a population-based vaccine effort against EBOV for the endemic areas in Africa [2]. Among the most promising candidates are adenovirus-based and modified vaccinia Ankara (MVA)-based vaccines [2].

Adenovirus (Ad)-based vectors have frequently been developed for emerging viral diseases; however, the high seropositivity in humans for human Ads limited their use. Chimpanzee adenovirus (ChAd)-EBOV was developed to circumvent these preexisting immunity complications associated with particularly human Ad5-based vaccines [2]. However, the ChAd-EBOV vaccine by itself as a single high-dose vaccine only elicits short-lived protective immune responses and requires a

boost vaccination [7]. Studies in NHPs have shown that a ChAd-EBOV prime together with a boost of MVA-based vaccines expressing the EBOV GP is effective in mediating long-term protective immunity and has been analyzed in several clinical trials [2]. A single dose of this MVA-EBOV GP vaccine was also not protective in NHPs [7], which is not unexpected considering previous approaches with a vaccinia virus-based vaccine resulted in partial protection in guinea pigs and failed to protect NHPs [8]. However, does MVA have the potential to being developed as an effective single dose vaccine against EBOV?

MVA is a highly attenuated vaccinia virus first produced in Germany in 1975 as a 'safer smallpox vaccine' for immunocompromised individuals considered to be at risk for the standard vaccinia inoculation [9,10]. Attenuation was accomplished by 570 serial passages in chicken embryo fibroblasts resulting in a virus that has lost about 30,000 bases of a wild-type vaccinia virus genome, particularly affecting genes important for virulence and immune evasion [8]. While MVA replicates well in avian cells, it undergoes an abortive infection in primates [11]. Despite its abortive infection in primates, MVA has retained desirable features of its vaccinia parent: the elicitation of durable T cell and antibody (Ab) responses [12], the ability to be stored as a lyophilized product at ambient temperature (www.ClinicalTrials.gov; NCT00914732), and the ability to be used without an adjuvant. Due to its large genome size and the amount of coding capacity lost during adaptation (~20%), MVA can be used as a vector to express multiple vaccine antigens [13], a characteristic that readily supports the construction of recombinant MVAs expressing foreign virus-like particles (VLPs) [14–16]. Recently, promise for a safer and more stable vaccine has been demonstrated using MVA to express EBOV-like particles to achieve single-dose protection in nonhuman primates [14]. The advantage of this particular MVA vector compared to previously used vectors only expressing EBOV GP is that it expresses two EBOV antigens – the GP and the matrix protein VP40. Expression of both EBOV proteins from a MVA-infected cell leads to EBOV-like particle formation and the generation of a protective immune response [14,15]. The single-dose protection in nonhuman primates recently reported in Nature's Scientific Reports reflects this 'first in class' VLP vaccine benefitting

from the choice of insertion sites and promoters and the careful design of the EBOV GP and VP40 transgenes used in its construction [14]. Domi et al. reported uniform protection of the MVA-EBOV vaccine when a single-dose was administered 28 days prior to lethal challenge with EBOV. The animals developed good humoral immune responses including antigen-specific IgG and EBOV-neutralizing antibodies [14].

The challenge now is how one takes forward a vaccine such as MVA-EBOV, with preclinical protective potential, but potentially improved safety, durability, and stability profiles than a vaccine that has successfully completed an initial efficacy test (VSV-EBOV). Does the safety and stability during storage of this vaccine merit the cost to develop this vaccine for developing countries? In the 5643 adults and 194 children inoculated in the VSV-EBOV ring vaccination study, there was only 1 severe adverse event, a febrile episode, that was judged to be causally related to vaccination [3]. Compared to the Yellow fever vaccine, which can lead to severe complications [5], that is very little and warrants the continued use of this vaccine particularly in emergency situations. However, for travelers and health care workers from the developed world a safer replication-incompetent vaccine with no associated adverse effects, which might even require more than one vaccination dose, would be of value as these individuals can plan for vaccination before exposure. The military also would benefit from a safer prime-boost vaccine approach with no associated adverse effects, especially one that could protect against aerosol infections, considering that filoviruses are potential biological terrorism agents [17]. In some studies, VSV-EBOV has protected against an aerosol EBOV challenge, the likely form of a weaponized challenge [18]. Thus, the next most important step for the MVA-EBOV vaccine is to test its potential, and the reproducibility of its potential, to protect against an aerosol challenge. Another development pathway will be to evaluate the ability to formulate the MVA-EBOV vaccine into a multi-hemorrhagic fever virus vaccine for citizens of West and Central Africa, travelers, first responders to outbreaks, and the military. The goal for such a vaccine would be to protect against EBOV, Sudan (SUDV), Bundibugyo, and Marburg virus (MARV), the four highly lethal filoviruses that have caused at least 29 outbreaks since 1976 in Central and West Africa. Additionally, one should also include Lassa virus (LASV), an arenavirus, in this vaccine, as it is endemic to West Africa and the causative agent of thousands of cases of highly lethal hemorrhagic fever each year. Ebolaviruses do not induce cross-reactive immune responses between species or even to MARV. It will be challenging to formulate a vaccine of that many components protective against these different pathogens, regardless if it will be a blend of single MVA-VLP vaccines or one single MVA-vector expressing multiple antigens. However, for the latter strategy, a multivalent VSV vector against EBOV, SUDV, and MARV has been developed and shown to be efficacious in rodents [19]. Alternatively, consecutive vaccinations with MVA-VLP vectors targeting different pathogens could be considered; however, the effect of preexisting immunity targeting MVA for such a strategy needs to be evaluated in preclinical studies. This has been shown to be a viable option for the VSV-based vaccines, particularly for the VSV-EBOV and VSV-LASV vaccines [20]. Consecutive vaccinations enable the use of all VSV-based

vaccines for EBOV, LASV, MARV, and other pathogens endemic in West and Central Africa in one host. In studies in nonhuman primates, VSV vaccines can be successfully used in previously immunized individuals.

The MVA-VLP vaccine platform has high versatility and is currently being developed not only for the pathogens mentioned earlier, but also for members of unrelated virus families like human immune deficiency virus, Zika virus (ZIKV), Hepatitis B virus, and even the parasites of the species *Plasmodium sp.*, the causative agents of malaria. Furthermore, every effort is being made to advance the MVA-EBOV and the MVA-ZIKV vaccines, which target two recent emerging pathogens, toward licensure.

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Declaration of interest

HLR is co-founder and owns stock in GeoVax, Inc., the developer of the MVA-EBOV like particle vaccine. The authors have no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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ORCID

Harriet L. Robinson  <http://orcid.org/0000-0001-6380-3458>
 Andrea Marzi  <http://orcid.org/0000-0003-0186-9587>

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