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REVIEW

Ebola vaccines in clinical trial: The promising candidates

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ABSTRACT

Ebola virus disease (EVD) has become a great threat to humans across the world in recent years. The 2014 Ebola epidemic in West Africa caused numerous deaths and attracted worldwide attentions. Since no specific drugs and treatments against EVD was available, vaccination was considered as the most promising and effective method of controlling this epidemic. So far, 7 vaccine candidates had been developed and evaluated through clinical trials. Among them, the recombinant vesicular stomatitis virus-based vaccine (rVSV-EBOV) is the most promising candidate, which demonstrated a significant protection against EVD in phase III clinical trial. However, several concerns were still associated with the Ebola vaccine candidates, including the safety profile in some particular populations, the immunization schedule for emergency vaccination, and the persistence of the protection. We retrospectively reviewed the current development of Ebola vaccines and discussed issues and challenges remaining to be investigated in the future.

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Introduction

Ebola virus was first identified in Central Africa with 2 simultaneous outbreaks in Sudan and Zaire (now Democratic Republic of the Congo) separately in 1976, and then be named after the Ebola River in Zaire.[1] Ebola virus is an enveloped, single stranded, negative sense RNA virus, which comprises 5 distinct subspecies: Zaire Ebola virus (EBOV), Sudan Ebola virus (SUDV), Bundibugyo Ebola virus (BDBV), Tai Forest Ebola virus (TAFV) and Reston Ebola virus (RESTV).[2] Among them, EBOV is the most dangerous subspecies which cause a high case fatality among human and non-human primates (NHPs).[3-5] Ebola virus could lead to EVD, formerly known as Ebola hemorrhagic fever,[6] which is a severe acute viral illness and often characterized by the sudden onset of fever, weakness, headache, muscle pain, sore throat, hiccups conjunctivitis, red eyes, rash, diarrhea, vomiting, internal and external bleeding.[6] Ebola virus can transmit from animals to humans and then spread among human beings quickly through closely contacts with the infected blood, bodily fluids or tissues. Moreover, the release of Ebola virus by small-particle aerosol dispersion would probably result in mucosal infection.[6] Due to the high level of infectivity and severity, Ebola virus has been listed as Biosafety Level-4 Virus by World Health Organization (WHO).

The 2014 West Africa outbreak is the largest and the most serious Ebola virus outbreak in history, with an approximate total reported cases count of 28646 and 11323 deaths till 27 March, 2016.[7] This EVD outbreak had spread from Guinea into Liberia, Nigeria, Senegal, Sierra Leone and Mali, with individual case exportations or transport of patients to France, Germany, Norway, Spain, United Kingdom (UK), and United States (US), causing a worldwide alarm.[6] Since there were no specific drugs or treatments for EVD, vaccination was considered as a most efficient method to control the spreading of Ebola virus. Several Ebola vaccine development campaigns were swiftly launched for clinical trials in order to cope with the Ebola epidemics.[8] In this review, we aim to provide an overview of the current research and development of vaccine candidates against Ebola virus, including the efficacy, immunogenicity, and safety profiles of the vaccines, and discuss further research topics and directions in the future.

Structural and functional characteristics of Ebola

The mature Ebola virion is comprised of 2 main components that nucleocapsid and envelope. The glycoprotein (GP) spikes form on the surface of envelope and the matrix consisted of virion protein (VP) 24 and VP40 locates in the middle of nucleocapsid and envelope (Fig. 1A). The Ebola virus genome is 19kb in size and comprised of 7 non-segmented genes, which encodes the nucleoprotein (NP), VP35, VP40, GP, VP30, VP24 and RNA-dependent RNA polymerase (L), respectively[9] (Fig. 1B). The viral RNA genome is encapsidated by the NP and together with L, the L cofactor VP35 and the transcriptional activator VP30, as well as VP24, which form a central nucleocapsid in the virus particle.[10] The structure of Ebola virus NP, VP35, VP30 and L are responsible for replication and transcription of viral RNA, while VP40 and VP24 are responsible for assembly, budding and release of virion particles.

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/khvi.
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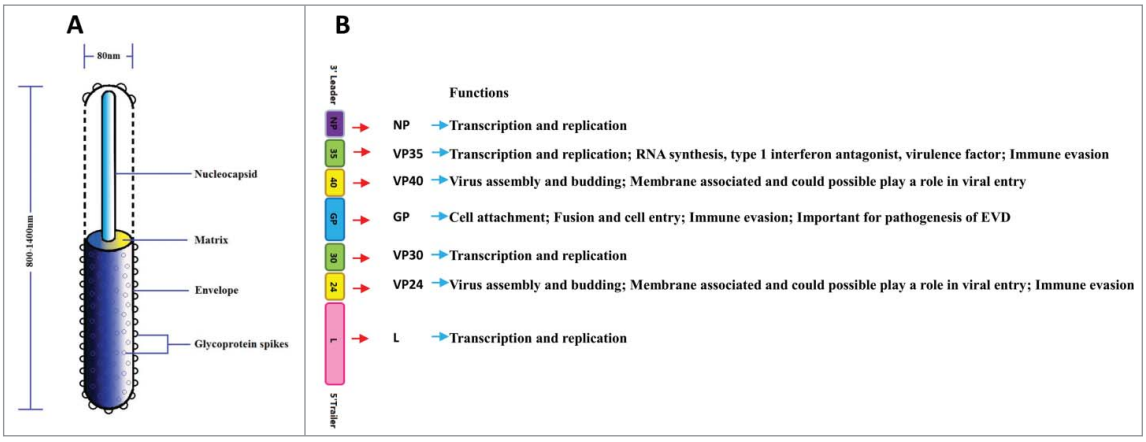

Figure 1. Structure and functions of Ebola virus and Ebola virus genes. (A) Shown are the structure of Ebola virus which comprised of two main factors that nucleocapsid and envelope. In the middle of nucleocapsid and envelope is matrix which is comprised of VP40 and VP24. Glycoprotein spikes are located on the surface of envelope. (B) Shown are schematic representations of Ebola virus genome which is comprised of seven non-segmented genes. These seven genes encode the nucleoprotein, virion protein35, VP40, glycoprotein, VP30, VP24 and RNA-dependent RNA polymerase.

Furthermore, VP35 and VP24 are implicated in immune evasion by blocking interferon (IFN) production and signaling. The former blocks detection of the dsRNA stage of viral replication/transcription, while the latter blocks a number of IFN signaling pathways.[11] The surface GP is a multimer of a single structural GP, which is responsible for cell attachment, fusion and cell entry, helps in immune evasion and plays a role in pathogenesis of disease.[8] The role of the viral GP makes it a key antigenic target for designing new Ebola vaccine candidates and immunotherapies.

Overview of the development of Ebola vaccines

The research on Ebola vaccines had already started in 1980 after the first discovery of Ebola virus. However, most of the researches were still in the animal study stages before 2014, and no evidence of protection in human beings had been obtained. Results from pre-clinical trial studies indicated that both humoral and cellular immunity play an essential role in controlling and eliminating virus due to the spiking of Ebola virus envelope GP forms on the surface of mature virions.[12-14]

The 2014 Ebola epidemic has significantly accelerated the development of Ebola vaccines, 46 clinical trials with Ebola vaccines were launched according to the registration on *Clinicaltrial.gov* and *Pan African Clinical Trials Registry* since then (Tables 1 and 2).[15] Different kinds of Ebola vaccines had been developed and evaluated, which can be roughly divided into 3 categories: non-replicative vector-based Ebola vaccines, replicative vector-based Ebola vaccines and others (Fig. 2). Non-replicative vector-based Ebola vaccines are those vaccines based on adapted vectors encoding the GP or other antigens of Ebola with deletions of genes essential for the life cycle of the vector virus to restrict the transcription and replication, including modified vaccinia strain Ankara (MVA)-vectored vaccines, venezuelan equine encephalitis virus (VEEV)-like replicon particles vaccine, human adenovirus vector-based Ebola vaccines, replication-defective recombinant chimpanzee adenovirus type 3-vectored vaccine (ChAd3 vaccine) and Kunjin replicon virus-like particle vaccine (KUN VLPs). Though the non-replicative vector-based Ebola vaccines were considered to have a better tolerability profile without causing a viremia after vaccination, high dosage of viral particles (vp) were needed to elicit a significant response. While the replicative vector-based Ebola vaccines could encode Ebola antigens with replicative vectors which were highly efficient with relatively low dosage, including rVSV-EBOV, human parainfluenza virus type 3-based vaccine (HPIV3), recombinant cytomegalovirus (rCMV)-based vaccine and recombinant rabies virus (RABV)-based vaccine. However, there are still some safety concerns associated with the replicative vector-based vaccines. Other Ebola vaccines included inactivated Ebola vaccine, DNA vaccine encoding the GP from EBOV and SUDV, virus-like particles (VLPs) and recombinant EBOVΔVP30 (rEBOVΔVP30). The timeline of the development of various Ebola vaccines were showed in Fig. 3. The rVSV-EBOV, ChAd3 vaccine, Ad26-EBOV, Ad5-EBOV, HPIV3, DNA vaccine and MVA-vectored vaccine have been evaluated in clinical trials, and the rVSV-EBOV has successfully showed a high protection against EVD from the preliminary results of a phase III trial conducted in West Africa.

Non-replicative vector-based Ebola vaccines

VEEV-like replicon particle vaccines

VEEV is a positive-sense RNA virus.[16] By replacement of VEEV structural protein genes, the Ebola NP or GP gene was packaged into a recombinant propagation-deficient VEEV replicon particles (VRP) expressed from an RNA expression vector and defined as NP-VRP or GP-VRP, respectively.[17] Guinea pigs were inoculated subcutaneously with a total of 0.5 ml containing 10^7 infectious units (IU)/ml of VRP then challenged subcutaneously with 1000 LD_{50} (10^4 plaque-forming units (PFU)) of guinea pig-adapted Ebola virus, while BALB/c mice were inoculated subcutaneously with 0.2 ml containing 10^6 IU of VRP then challenged intraperitoneally with 30-300 LD_{50} (1-10 PFU) of mouse-adapted Ebola virus.[17] Complete protection was noticed in both mice and guinea pigs after the vaccination of GP-VRP alone, or in combination with NP-VRP. (Table 3) By contrast, immunization with NP-VRP alone protected mice, but not guinea pigs. Besides, another study proved that 75-80% C57BL/6 mice vaccinated with 2×10^6 focus-forming units

Table 1. List of clinical trials which are single use of promising candidates.

Single Use Candidate Vaccines	Current Status	Sponser	Start Date	Phase	Estimated Enrollments	Ages	Locations	ClinicalTrials.gov Identifier
Ad5-EBOV	Completed	NIAID	Sept.2010	1	48	18-50	USA	NCT00374309
Ad5-EBOV	Completed	JSCDC	Dec.2014	1	120	18-60	China	NCT02326194
	Completed	JSCDC	Jul.2015	1	110	18-60	China	NCT02533791
	Completed	FAHZU	May.2015	1	61	18-60	China	NCT02401373
	Ongoing	JSCDC	Oct.2015	2	500	18-50	Sierra Leone	NCT02575456
DNA	Completed	NIAID	Oct.2003	1	27	18-44	USA	NCT00072605
	Completed	NIAID	Jan.2008	1	20	18-60	USA	NCT00605514
	Completed	NIAID	Fre.2010	1	108	18-50	Uganda	NCT00997607
rVSV-EBOV	Completed	Merck Sharp & Dohme Corp	Oct.2014	1	120	18-65	USA	NCT02280408
	Completed	Merck Sharp & Dohme Corp	Oct.2014	1	39	18-50	USA	NCT02269423
	Completed	Dalhousie University	Nov.2014	1	40	18-65	Not Provided	NCT02374385
	Completed	UHE	Nov.2014	1	30	18-65	Germany	NCT02283099
	Completed	Merck Sharp & Dohme Corp	Dec.2014	1	512	18-60	USA	NCT02314923
	Completed	University Hospital, Geneva	Dec.2014	1 & 2	115	18-65	Switzerland	NCT02287480
	Ongoing	University of Oxford	Dec.2014	1	40	18-55	Kenya	NCT02296983
	Ongoing	Novavax	Feb.2015	1	230	18-50	Australia	NCT02370589
	Ongoing	CDC	Apr.2015	2 & 3	8000	18+	Sierra Leone	NCT02378753
	Ongoing	Merck Sharp & Dohme Corp	Aug.2015	3	1198	18-65	USA, Canada, Spain	NCT02503202
	Ongoing	Profectus BioSciences, Inc.	Jan.2016	1	38	18-60	USA	NCT02718469
	Not recruit	NIAID	Jan.2016	2	300	18+	USA	NCT02788227
cAd3-EBO	Completed	CHUV	Oct.2014	1 & 2	120	18-65	Switzerland	NCT02289027
	Ongoing	GSK	Jul.2015	2	2796	18+	Senegal	NCT02485301
cAd3-EBO/ChAd3-EBO-Z	Ongoing	NIAID	Aug.2014	1	50	18-65	USA	NCT02231866
HPIV3-EBO-Z	Ongoing	NIAID	Aug.2015	1	30	18-50	USA	NCT02564575

Abbreviations: NIAID = National Institute of Allergy and Infectious Diseases; JSCDC = Jiangsu Province Centers for Disease Control and Prevention FAHZU = First Affiliated Hospital of Zhejiang University; NLGC = NewLink Genetics Corporation; UHE = Universitätsklinikum Hamburg-Eppendorf; CDC = Centers for Disease Control and Prevention; CHUV = Center Hospitalier Universitaire Vaudois; GSK = GlaxoSmithKline; USA = United States.

(FFU) of NP-VRP survived lethal challenge with 10 PFU of mouse-adapted Ebola virus intraperitoneally.[18] On the basis of the protection observed in rodents challenge model, another study demonstrated that cynomolgus macaques can also be completely protected from the lethal challenge with 1000 PFU of Ebola virus after administrating with one single dose 10^{10} FFU of GP-VRP intramuscularly, which lighted the potential of VEEV Ebola vaccines.[19]

Adenovirus vector-based Ebola vaccines

In 2000, Sullivan et al.[20] first used a recombinant replication defective human adenovirus 5 (rAd5)-vectored Ebola vaccine, which was generated by introducing gene of EBOV-GP into the rAd5 full-length plasmid. NHPs was prime immunized with DNA vaccine at week 0, 4 and 8, then boosted with the rAd5-based Ebola vaccine at week 32, resulting in a 100% protection against the lethal challenge with 6 PFU of Ebola virus intraperitoneally. Considering the long immunization schedule could not meet the requirement of emergency immunization in Ebola outbreak, they further vaccinated NHPs with a single dose of an improved rAd5-EBOV containing both GP and NP antigens, which also achieved a complete protection in NHPs (Table 3).[21] However, the prior prime-boost strategy may provide a greater durability and efficacy than a single injection of rAd5-EBOV. Besides, CD8+ cells were found to play a major role in rAd5-GP-induced immune protection against EBOV infection in NHPs.[22]

In 2010, Ledgerwood et al.[23] conducted the very first human clinical trial with a rAd5-based Ebola vaccine developed by Crucell Holland BV in healthy adults at National Institutes of Health (NIH) Clinical Center. This was a randomized, double-blinded, placebo-controlled, and dose-escalating phase I trial (ClinicalTrials.gov NCT00374309) (Table 1). The rAd5-based Ebola vaccine encoding both the GPs of the EBOV (Kikwit 1995) and SUDV subspecies, which demonstrated a 100% protection in previously assessed NHPs challenging models.[24] 31 healthy adults were allocated randomly to receive intramuscular injection of either rAd5-based Ebola vaccine at 2×10^9 vp, or 2×10^{10} vp or placebo. The results showed that this rAd5-based Ebola vaccine was able to elicit both specific humoral and cellular immune responses, and the most common adverse reaction is mild and short-lived headache. Both the low dose and high dose vaccines were well tolerated, while the Ebola GP-specific antibody titers and the T-cell responses were significant greater in the high-dose groups. However, a critical concern of the human Ad5 vector-based vaccines was the commonly existed pre-existing immunity (PEI) to human Ad5 with a baseline positive rate of 60-90% in the populations, which may compromise the effectiveness of the Ad5 vectored vaccine in inducing humoral and cellular immunogenicity.[25] Thus some scientists had tried to replace the human Ad5 vector with other less common human adenovirus such as Ad26 and Ad35, which exhibited a low pre-existing antibody in humans.[26-28] In 2011, a NHPs challenging study with a prime-boost regimen using heterologous Ad26-vectored and Ad35-vectored Ebola vaccines demonstrated a significant protection after challenging with 1000 PFU of EBOV[28] (Table 3). In addition, Ad35 and Ad26-vectored vaccines could induce potent antibody and T-cell responses to multiple filovirus species.[29] Currently, the Ad26-EBOV vaccine has been evaluated in clinical trials (Table 2). A single-center, randomized, placebo-controlled, observer-blind, phase I trial adopting prime-boost regimen (prime with Ad26-EBOV or MVA-BN Filo and boost with the alternative vaccine 28 or 56 d later) enrolled 87 participants to evaluate the safety and immunogenicity of the Ad26-EBOV (Table 4).[30] Mild to moderate injection-site pain was the

Table 2. List of clinical trials which are combined use of promising candidates.

Combine Use Candidate Vaccines	Current Status	Sponser	Start Date	Phase	Estimated Enrollments	Ages	Locations	ClinicalTrials.gov Identifier
Ad26-ZEBOV+MVA-BN Filo	Completed	Crucell Holland BV	Dec.2014	1	88	18-50	UK	NCT02313077
	Ongoing	Crucell Holland BV	Jan.2015	1	164	18-50	USA	NCT02325050
	Ongoing	Crucell Holland BV	Apr.2015	1	78	18-50	Uganda;Tanzania	NCT02376400
	Ongoing	Crucell Holland BV	Mar.2015	1	72	18-50	Ghana; Kenya	NCT02376426
	Recruiting	Crucell Holland BV	Jun.2015	2	612	18-65	France, UK	NCT02416453
	Recruiting	Crucell Holland BV	Sept.2015	3	525	18-50	USA	NCT02543567
	Recruiting	Crucell Holland BV	Sept.2015	3	728	1-65	Sierra Leone	NCT02509494
	Recruiting	Crucell Holland BV	Oct.2015	2	1188	1-70	Africa countries	NCT02564523
	Ongoing	Crucell Holland BV	Sept.2015	3	329	18-50	USA	NCT02543268
	Recruiting	Crucell Holland BV	Jan.2016	2	575	18-70	USA,Kenya,Nigeria	NCT02598388
	Recruiting	Crucell Holland BV	Jan.2016	4	5500	1-71	Not Provided	NCT02661464
Ad26.Filo+MVA-BN Filo	Not recruit	Janssen Vaccines & Prevention B.V.	Aug.2016	1	72	18-50	USA	NCT02860650
ChAd3-EBO-Z+MVA-BN Filo	Recruiting	University of Oxford	Dec.2014	1	92	18-50	USA	NCT02240875
	Ongoing	University of Maryland	Nov.2014	1	91	18-50	Mali	NCT02267109
ChAd3-EBO-Z+MVA-ZEBOV	Completed	University of Oxford	Jul.2015	1	40	18-50	Senegal	NCT02485912
	Ongoing	University of Oxford	Apr.2015	1	38	18-50	UK	NCT02451891
cAd3-EBO+MVA-ZEBOV	Ongoing	University of Maryland	May.2015	1	60	18-65	Mali	NCT02368119
	Ongoing	NIAID	Mar.2015	1	64	18-66	USA	NCT02408913
cAd3-EBO/ChAd3-EBO-Z+MVA-ZEBOV	Ongoing	NIAID	Jan.2015	1	90	18-65	Uganda	NCT02354404
ChAd3-EBO-Z+Ad26-ZEBOV	Ongoing	University of Oxford	Sept.2015	1	32	18-50	UK	NCT02495246
ChAd3-EBO-Z+rVSV-EBOV	Ongoing	NIAID	Jan.2015	2	28170	18+	Liberia	NCT02344407
ChAd3-EBO-Z+Nimenrix	Ongoing	GSK	Nov.2015	2	600	1-17	Not Provided	NCT02548078

Abbreviations: NIAID = National Institute of Allergy and Infectious Diseases; GSK = GlaxoSmithKline; USA = United States; UK = United Kingdom.

most commonly reported adverse event. Though 4 serioues adverse events occurred, none of these were considered related to the experimental vaccines. According to the report, more than 90% of vaccinees generated Ebola GP-specific IgG 4 weeks after a priming dose of Ad26-EBOV, and 55% developed specific T cells. Furthermore, responses were enhanced by administration of an MVA-BN Filo booster dose and were sustained at 8 months after the prime vaccination. The immunogenicity and safety of these vaccines are being further assessed in phase II and III studies. These results hinted that choosing a vector with low pre-existing antibody in human may become an approach to solve PEI.

Even though there were several questions or doubts about the Ad5-based vaccine, the development of the Ebola vaccine based on the Ad5 vector did not stop. After the 2014 Ebola outbreak, a novel recombinant human Ad5 vector based Ebola vaccine (Ad5-EBOV) expressing the GP of the 2014 epidemic Ebola strain (Guinea, 2014) was jointly developed by Beijing Institute of Biotechnology and Tianjin CanSino Biotechnology Inc.[31] Two outstanding advantages of this Ad5-EBOV was noticed: first, the Ad5-EBOV is the first Ebola vaccine developed according to the 2014 epidemic strain, which was considered to be a new epidemic strain, with 96.7% homology of the nucleotide sequence and 97.6% homology of amino acid sequence[31] compared to the GP gene of the strain in 1976 which was based on by other vaccines;[32,33] Second, the Ad5-EBOV is lyophilized white powder (can be stored at 2-8°C), which may be more

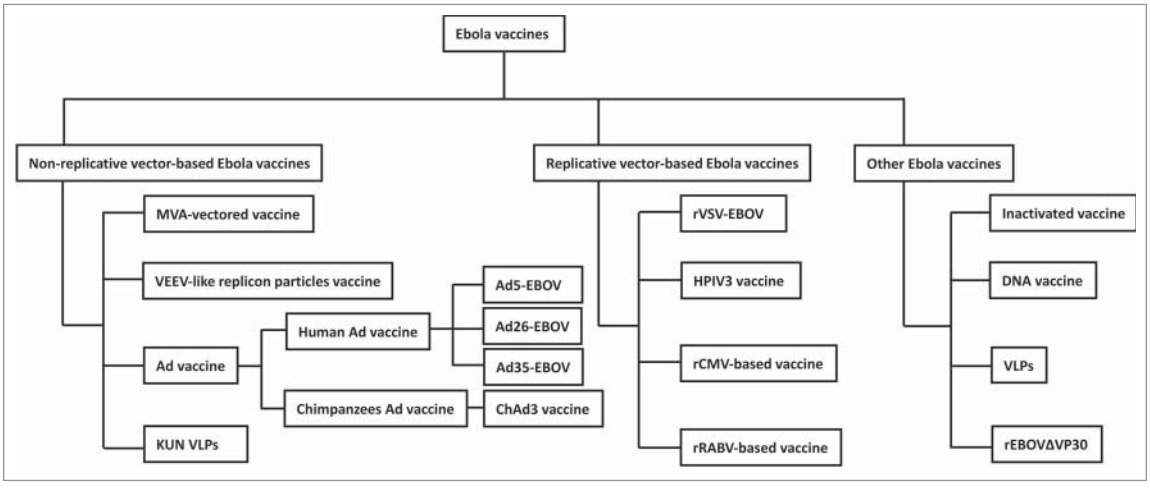

Figure 2. Classification of Ebola vaccines. Ebola vaccines can be roughly divided into three classifies that non-replicative vector-based Ebola vaccines, replicative vector-based Ebola virus vaccines and other Ebola vaccines.

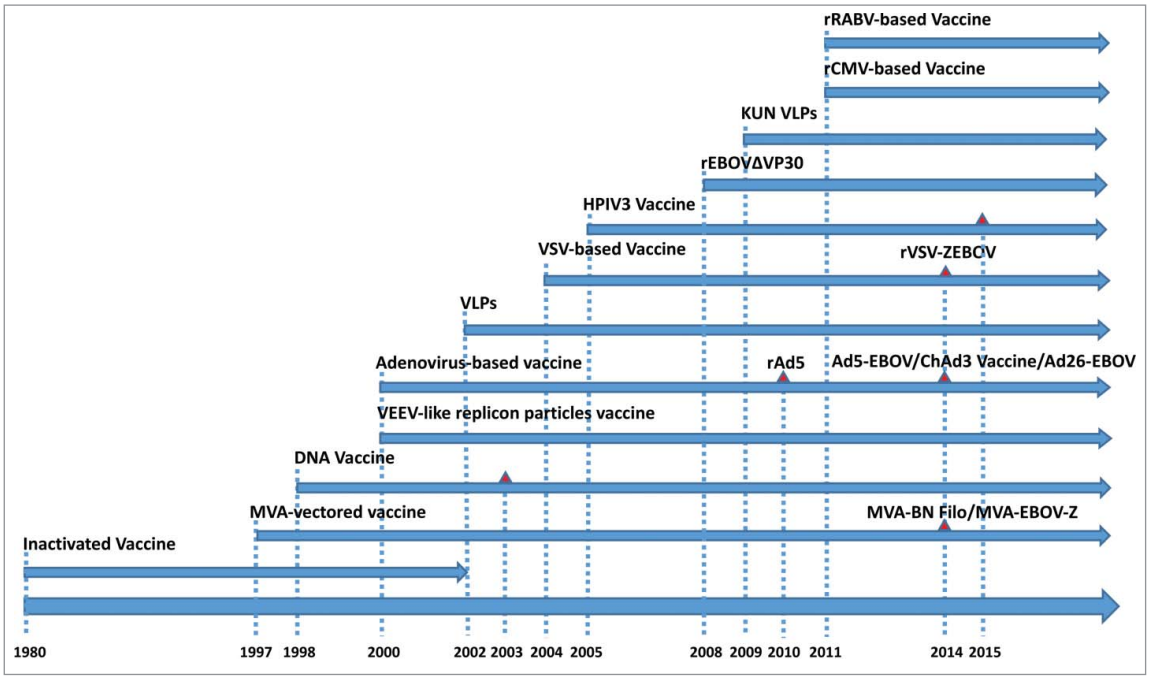

Figure 3. Development history of Ebola virus vaccines. Shown are schematic representations of the sequence of development of Ebola virus vaccines and the start time of clinical trials. Each line represents the development of a kind of vaccine with its name ahead. The red triangle represents the start point of clinical trials of promising candidate. Almost every vaccine continues research up to date except inactivated vaccine which was stopped in 2002.

suitable for the areas where the cold chain system is incomplete than those liquid formulations.

After a preliminary efficacy was observed in the pre-clinical animal studies, the Ad5-EBOV was quickly put into the clinical trials at the end of 2014 (Table 1). The safety, tolerability and immunogenicity of the Ad5-EBOV was evaluated in 120 healthy adults in China (ClinicalTrials.gov NCT02326194), receiving either one shot of the Ad5-EBOV at 4×10^{10} vp, 1.6×10^{11} vp, or placebo.[31] The safety observation for adverse reactions post-vaccination indicated a good safety profile of the Ad5-EBOV, which was in line with the previous reports of other Ad5-based Ebola vaccines. Though higher incidence of injection-site reactions was associated with the higher dosage of Ad5-EBOV, most of the reactions were mild or moderate. Ebola GP-specific antibody titers were significantly increased in participants in both the 4×10^{10} vp and 1.6×10^{11} vp Ad5-EBOV groups with a geometric mean titer (GMT) of 421.4 and 820.5 at day 14, and 682.7 and 1305.7 at day 28, respectively. Moreover, T-cell responses peaked at day 14. The magnitude of both humoral and cell responses were greater in participants with low or negative pre-existing Ad5 neutralizing antibody than in those with high pre-existing Ad5 antibody (Table 4). The results indicated that the high-dose 1.6×10^{11} vp Ad5-EBOV could overcome the negative effects of PEI and still induced robust Ebola GP-specific antibody and T-cell responses.

In May 2015, another single-center, open-label phase I clinical trial was conducted, recruiting 60 healthy African adults in China (ClinicalTrials.gov NCT02401373), to further assess the safety profile of the experimental Ad5-EBOV in Africans. Participants were allocated into 2 groups to receive either low dose (4×10^{10} vp) or high dose (1.6×10^{11} vp) vaccine, while the particular data is unpublished (Table 1).

In October 2015, a phase II clinical trial of the experimental Ad5-EBOV was launched and ongoing in Sierra Leone, West Africa (ClinicalTrials.gov NCT02575456). A total of 500 healthy local adults were recruited and randomly allocated to receive one dose of 1.6×10^{11} vp, 8×10^{10} vp, or placebo at a ratio of 2:1:1 to further evaluate the safety and immunogenicity of Ad5-EBOV (Table 1).

ChAd3 vector was considered as a replacement of the human Ad5 vector for Ebola vaccine in order to cope with the PEI against Ad5. ChAd3 vectored Ebola vaccines can be divided into 2 kinds, one is the monovalent recombinant chimpanzee adenovirus type 3-vectored vaccine expressing wild-type GP from EBOV (ChAd3-EBO-Z), the other one is the bivalent recombinant chimpanzee adenovirus type 3-vectored vaccine expressing wild-type GP from EBOV or/and SUDV (cAd3-EBO). Stanley et al.[34] immunized cynomologous macaques with a single inoculation of 1×10^{11} or 1×10^{10} vp of ChAd3-EBO-Z and a protection against EBOV was noticed 5 weeks after challenging with 1000 PFU of EBOV (Table 3). Then they added SUDV GP into vaccine to advance the diversity of protection in a natural outbreak setting. The team immunized 4 macaques with cAd3-EBO and challenged them with a lethal dose of EBOV 5 weeks after vaccination. As seen with the monovalent vaccine, the bivalent vaccine also protected the macaques from infection demonstrating that inclusion of an additional GP species did not interfere with the protection from EBOV observed with the monovalent vaccine. However, the humoral and cellular immunity elicited by ChAd3 vaccine waned gradually over time and lost protection for NHPs from challenging 10 months after vaccination. Even in the NHPs inoculated of 1×10^{11} vp of ChAd3 vaccine, only a 50% protection was observed 10 months later. Thus, the research team attempted to adopt a prime-boost immune strategy: macaques was immunized with a prime dose of 1×10^{10} vp ChAd3 vaccine, and then a booster dose of ChAd3 vaccine,

Table 3. Comparison of Ebola vaccine candidates in animal studies.

Vaccine	Animal Model	Doses	Origin of antigen	Challenge Dose/Manner	Protection	ref
VEEV	Guinea pig	10^7 IU	EBOV (1976 Mayinga)	1000LD_{50} (10^4PFU) of guinea pig-adapted Ebola virus/S.C.	100%	17
	Mice	10^6 IU	EBOV (1976 Mayinga)	30-300 LD_{50} (1-10 PFU) of mouse-adapted Ebola virus/I.P.	100%	17
	Mice	2×10^6 FFU	EBOV (1995 Kikwit)	300 LD_{50} (10 PFU) of mouse-adapted Ebola virus/I.P.	75-80%	18
	NHPs	10^{10} FFU	EBOV (1995 Kikwit)SUDV (Boniface)	887-1050 PFU of SUDV; 943-1012 PFU of EBOV/I.M.16-132 PFU of SUDV/Aerosol	100%	19
DNA+Ad5	NHPs	10^{10} FFU	EBOV (1976 Mayinga)SUDV ; Ivory Coast	6 PFUs of EBOV/I.P	100%	20
Ad5	NHPs	2×10^{12} vp	EBOV (1995 Kikwit)	10/1000 PFU of EBOV/I.M.	100%	21
	NHPs	10^{10}vp	EBOV (1995 Kikwit)	1000 PFU of EBOV/I.M.	100%	24
Ad26+Ad35	NHPs	10^{10}vp	EBOV (1995 Kikwit)	1000 PFU of EBOV/I.M.	100%	28
ChAd3	NHPs	10^{10}vp	EBOV (1995 Kikwit)SUDV	1000 PFU of EBOV/I.M.	0%	34
ChAd3		10^{11}vp			50%	
ChAd3+ChAd3		10^{10}vp/10^{10}vp			33%	
ChAd3+ChAd63		10^{10}vp/10^{10}vp			25%	
ChAd3+MVA		10^{10}vp/10^8vp			100%	
MVA	NHPs	—	EBOV (1995 Kikwit)	1000 PFU of EBOV/I.M.	0%	41
KUN VLPs	Guinea pig	10^6/5×10^6 vp	EBOV (1976 Mayinga)	200 LD_{50} of guinea pig-adapted EBOV/I.P.	25–75%	43
	NHPs	10^9vp	EBOV (1976 Mayinga)	600 PFU of EBOV/I.M.	75%	44
VSV	Mice	2×10^4 PFU	EBOV (1976 Mayinga)MARV (1980 Musoke);Lassa virus(Josiah)	1000 LD_{50} of mouse-adapted Ebola virus/I.P.	100%	45
	NHPs	10^7PFU	EBOV (1976 Mayinga)MARV (1980 Musoke);	1000 PFU of EBOV/I.M.	100%	46
	Mice	2×10^5 PFU	EBOV (1976 Mayinga)MARV	1000 PFU of mouse-adapted EBOV/I.P.	100%	47
	Guinea pig	2×10^5 PFU	(1980 Musoke);Lassa virus(Josiah)	1000 PFU of guinea pig-adapted EBOV/I.P.	50%	
	NHPs	2×10^7 PFU		1000 PFU of EBOV/I.M.	50%	
HPIV3	Guinea pig	$10^{5.3}$PFU	EBOV (1976 Mayinga)	1000 PFU of guinea pig-adapted EBOV/I.P.	100%	60
	NHPs	4×10^6 /2×10^7 PFU	EBOV (1976 Mayinga)	1000 PFU of EBOV/I.M.	100%	9
	Guinea pig	4×10^5 /4×10^6 PFU	EBOV (1976 Mayinga)	1000 PFU of guinea pig-adapted EBOV/I.P.	100%	62
rCMV	Mice	10^5/5×10^5 PFU	EBOV (1976 Mayinga)	1000 PFU of mouse-adapted EBOV/I.P.	100%	63
rRABV	Mice	10^5PFU	EBOV (1976 Mayinga)	1000 PFU of mouse-adapted EBOV/I.P.	100%	68
	Mice	5×10^5 FFU	EBOV (1976 Mayinga)	1000 PFU of mouse-adapted EBOV/I.P.	100%	69
	NHPs	5×10^7 FFU	EBOV (1976 Mayinga)	1000 PFU of EBOV/I.M.	100%	70
Inactivated vaccine	Guinea pig	1.7×10^5 PFU	EBOV (1976 Mayinga)	10000 PFU of guinea pig-adapted EBOV/I.P.	100%	73
	Mice	1.4ug	EBOV (1995 Kikwit)	300 LD_{50} (10 PFU) of mouse-adapted Ebola virus/I.P.	100%	74
	NHPs	10^8 PFU	EBOV (1995 Kikwit)	1000 PFU of EBOV/I.M.	0%	
	NHPs	8.0 log10 PFU	EBOV (1995 Kikwit)	1000 PFU of EBOV/I.M.	0%	41
DNA	Mice	0.5ug+1.5ug	EBOV (1995 Kikwit)	30 LD50 of mouse-adapted EBOV/I.P.	100%	76
	Mice	5ug/20ug	EBOV (1995 Kikwit)	1000 PFU of mouse-adapted EBOV/I.P.	100%	77
VLPs	Mice	0.1/1/10ug	EBOV (1995 Kikwit)MARV	10/300 PFU of mouse-adapted EBOV/I.P.	100%	83
	NHPs	250ug	(1980 Musoke)	1000 PFU of EBOV/I.M.	100%	
rEBOVΔVP30	Mice	2×10^6 FFU	EBOV (1976 Mayinga)	1000 PFU of mouse-adapted EBOV/I.P.	100%	89
	Guinea pig	10^7FFU	EBOV (1976 Mayinga)	1000 PFU of guinea pig-adapted EBOV/I.P.	100%	
	NHPs	10^7 FFU	EBOV (1976 Mayinga)	1000 PFU of EBOV/I.M.	100%	90

Abbreviations: FFU, focus-forming units; PFU, plaque-forming units; vp, viral particles; EBOV, Zaire Ebola virus; SUDV, Sudan Ebola virus; MARV, Marburg virus; GMT, Geometric mean titer; GMC, Geometric mean concentration; NHPs, nonhuman primates; I.M., intramuscular; I.P., intraperitoneally; S.C., subcutaneously; infectious units, IU.

chimpanzee adenovirus type 63-vectored Ebola vaccine (ChAd63-EBO), or multivalent MVA-vectored vaccine (MVA-BN Filo) 8 weeks later.[34] The prime-boost regimen with ChAd3 vaccine and MVA-BN Filo achieved a full protection and durable immunity at month 10 post-immunization after challenging with 1000PFU of EBOV. On the contrary, the other 2 regimens (ChAd3 vaccine+ChAd3 vaccine and ChAd3 vaccine+-ChAd63-EBO) provided a compromised protection to one third of NHPs or one fourth of NHPs (Table 3). These results from animal studies advanced development of the ChAd3 vaccine into clinical trials.

In September 2014, a phaseI, dose-escalation, open-label trial was conducted to evaluate safety, and immunogenicity of cAd3-EBO by enrolling 20 healthy adults into 2 sequentially groups of 10 each at dosage of 2×10^{10} vp or 2×10^{11} vp (ClinicalTrials.gov NCT02231866).[35] This trial found that both the reactogenicity and immunogenicity of the experimental cAd3-EBO were dose-dependent. The GP Zaire-specific antibody titer at week 4 in 2×10^{11} vp dose group was significant higher than that in the 2×10^{10} vp dose group (2037 in 2×10^{11} vp dose group vs 331 in the 2×10^{10} vp dose) (Table 4). The incidences and severity of local and systemic adverse reactions were similar to those observed in previous studies of other adenovirus vectored vaccines.[23,36]

Beside, another phaseI, dose-escalation, open-label study was conducted to assess the safety and immunogenicity of a single dose of the ChAd3-EBO-Z at 3 different dosages 1×10^{10}, 2.5×10^{10} and 5×10^{10} vp, with 20 participants per group, respectively[33] (ClinicalTrials.gov NCT02240875) (Table 1). The highest dosage of the experimental vaccines in this trial only a quarter of that in the previous trial. No safety concerns were identified at any of the dosage levels studied, majority of the recorded local and systemic adverse events was mild and short-lived. The experimental vaccine had

Table 4. Comparison of Ebola vaccine candidates in clinical trials.

Vaccine	Dosages	Origin antigens	Immune responses (EBOV GP)	Most common AE	Ref
Ad5	Low (2×10^9 vp) High (2×10^{10} vp)	EBOV(Kikwit,1995) SUDV(Gulu)	GMT: Low:85 (Day28) High:155 (Day28)	Headache	23
Ad5	Low (4×10^{10} vp) High (1.6×10^{11} vp)	EBOV(Guinea,2014)	GMT: Low:682.7 (Day28) High: 1305.7 (Day28)	Injection-site pain	31
Ad26+MVA	Group1Prime:Ad26 (5×10^{10} vp) Boost:MVA (10^8TCID$_{50}$) Group2 Prime: MVA (10^8TCID$_{50}$) Boost:Ad26 (5×10^{10} vp)	EBOV(Mayinga,1967) SUDV;MARV;TAFV	GMC:Group1:7553 (Day21) Group2:18474 (Day21)	Injection-site pain	30
ChAd3	Low (2×10^{10} vp) High (2×10^{11} vp)	EBOV(Mayinga,1967)SUDV(Gulu)	GMT: Low:331 (Day28)High: 2037 (Day28)	Fever	35
ChAd3+MVA	Prime: ChAd3 Group1 (1×10^{10} vp) Group2 (2.5×10^{10} vp) Group3 (5×10^{10} vp) Boost: MVA1.5×10^8 PFU 3×10^8 PFU	EBOV(Mayingaq,1967) SUDV;MARV;TAFV	GMT: Prime:758 (6 Month) Boost:1750 (6Month)	Injection-site pain	33/37
ChAd3	Group1 (2.5×10^{10} vp) Group2 (5×10^{10} vp)	EBOV(Mayinga,1967)	GMC:Group1:51μg/mL (Day28) Group2:44·9μg/mL (Day28)	Fatigue/Malaise	38
ChAd3+MVA	Prime:ChAd3Group1(1×10^{10} vp) Group2 (2.5×10^{10} vp) Group3 (5×10^{10} vp)Group4 (1×10^{11} vp)Boost: MVA (2×10^8 vp)	EBOV(Mayinga,1967) SUDV;MARV;TAFV	GMT: PrimeGroup1:295.0 (Day28)Group2:204.6 Day28) Group3:555.8 (Day28) Group4:1493.6 (Day28) Boost:9279.6 (Day28)	Injection-site pain	39
rVSV	Site1and2:Group1 (3×10^6 PFU) Group2 (2×10^7 PFU) Site3: Group1 (3×10^5 PFU) Group2 (3×10^6 PFU) Site4: Group1(1×10^7 PFU) Group2 (5×10^7 PFU)	EBOV(Kikwit,1995)	GMT:Site1:Group1:1392.9 (Day28)Group2:1969.8 (Day28) Site2:Group1:1492.9 (Day28) Group2: (—) (Day28)Site3: Group1:1055.6 (Day28) Group2:2570.9 (Day28)Site4: Group1:1064.2 (Day28) Group2:1780.1 (Day28)	Injection-site pain	53
rVSV	Group1 (3×10^5 PFU)	EBOV(Kikwit,1995)	GMT:Group1:344.5 (Day28)	Injection-site pain	54
rVSV	Group1 (3×10^6 PFU)Group2 (2×10^7 PFU)	EBOV(Kikwit,1995)	GMT:Group1:1300 (Day28) Group2:4079 (Day28)	Injection-site pain	55
rVSV	Group (2×10^7 PFU)	EBOV(Kikwit,1995)	—	Injection-site pain	56
DNA	Group1(2.0mg)Group2(4.0mg) Group3 (8.0mg)	EBOV(Mayinga,1967)SUDV	—	Local reactions	78
DNA	Prime: Group(4.0mg)Boost: Group (4.0mg)	EBOV(Mayinga,1967) SUDV;MARV	GMT:Group:31.8 (Day28) after 3rdGroup:34.0 (Day28) after 4th	Injection-site pain	79
DNA	Group (4.0mg)	EBOV(Mayinga,1967) SUDV;MARV	GMT:Group:31.0 (Day28) after 3rd	Injection-site pain	32

Abbreviations: TCID50, median tissue culture infective dose; PFU, plaque-forming units; vp, viral particles; EBOV, Zaire Ebola virus; SUDV, Sudan Ebola virus; MARV, Marburg virus; GMT, Geometric mean titer; GMC, Geometric mean concentration.

successfully induced both specific antibody and T-cell responses, but the immune response levels were lower than those induced in previous trial at a dosage of 2×10^{11} vp (Table 4). All these results indicated that the immune responses and antibody titers are highly dose-dependent.

Later, the team added a booster dose of MVA to access the effect in 30 of the 60 participants and evaluated a reduced prime–boost interval in another 16 participants.[37] At 3 to 10 weeks after the priming immunization, the team further administered 18 participants with 1.5×10^8 PFU of MVA vaccine, while at a dose of 3×10^8 PFU to 12 participants. Significant increases in neutralizing antibodies were seen after boosting in all 30 participants (GMT139). ChAd3-EBO-Z boosted with MVA elicited B-cell and T-cell immune responses to EBOV that were superior to those induced by the ChAd3-EBO-Z alone. Besides, the prime–boost intervals as short as 1 week, which may facilitate vaccine deployment in outbreak regions.

Between October 24, 2014, and June 22, 2015, a phase I/II study included 120 health participants randomly assigned into deployed and non-deployed groups to receive a single intramuscular dose of ChAd3-EBO-Z at 5×10^{10} vp, 2.5×10^{10} vp or placebo.[38] (ClinicalTrials.gov NCT02289027) Fatigue or malaise was the most common systemic adverse event and no serious adverse events were reported. GMC of IgG antibodies against Ebola GP peaked at 51 μg/mL in the high-dose group, 44·9 μg/mL in the low-dose group on day 28. A single dose was immunogenic in almost all vaccine recipients and the antibody response in the vaccine group was still significantly higher than that in the placebo group at 6 months (Table 4). This favorable safety profile provides a reliable basis to proceed with the following phase II/III efficacy trials in Africa.[38]

Since studies in NHPs had shown that immunogenicity and duration of high-level protection against challenge could be extended by administering a dose of MVA-BN Filo as a booster.[34] Another study of ChAd3-EBO-Z with MVA-BN Filo among Malian and US adults began in late November 2014[39] (ClinicalTrials.gov NCT02231866 and NCT02267109, respectively) (Table 2). It was the first time that ChAd3-EBO-Z was

administered to Africans participants. Participants were randomly allocated to receive one-dose of 1×10^{10} or 2.5×10^{10} or 5×10^{10} or 1×10^{11} vp, then boosted participants with one-dose 2×10^{8} vp of MVA-BN Filo on day 79-111 post-priming. Most adverse events were mild, without any unexpected serious adverse reactions related to the vaccine. After the prime vaccination, the GMTs peaked at day 28 and then decreased slowly through the next 12 weeks. After boosting with MVA-BN Filo, the GMT rapidly increased by 36 times and persisted at a high level. MVA-BN Filo boosting was well-tolerated and powerfully immunogenic, eliciting significant anamnestic anti-GP antibody and multi-functional CD8 and CD4 T-cell responses irrespective of the dosage of ChAd3-EBO-Z priming or the prime-boost interval (Table 4). Up to date, more and more clinical trials investigated on a priming-boosting regimen of combined usage of different Ebola vaccines are ongoing to determine a more efficient immunization schedule.

MVA-vectored vaccines

In 1997, Gilligan et al.[40] first found that MVA-vectored vaccine expressing Ebola virus VP24 was able to prolong the mean life-span of guinea pigs after the lethal challenge with Ebola Virus. Though this vaccine can achieve a protection of 60% for guinea pigs, it was failed in protecting cynomolgus macaques in challenge model despite the fact that the neutralizing antibodies against EBOV were detected after the vaccinations (Table 3).[41] However, a further study demonstrated 100% protection for NHPs receiving a priming dose of ChAd3 vaccine with subsequent boosting by MVA-vectored vaccine.[34] So far, there are 2 kinds of MVA-vectored vaccines which have already advanced into clinical trial. One is a multivalent MVA-BN Filo that encodes the GPs from EBOV, SUDV, Marburg virus (MARV), and a NP from TAFV. The other one is a monovalent MVA-vectored vaccine encoding only EBOV GP.

Four phase I clinical trials using a prime-boost regimen with a combination of the Ad26-EBOV vaccine and MVA-BN Filo vaccine are ongoing in different countries (NCT02325050, NCT02313077, NCT02376400, NCT02376426) (Table 2). Besides, 2 phase II random, single-blind and placebo-control trials using the same prime-boost regimen are also ongoing in France and Africa countries, respectively (ClinicalTrials.gov NCT02416453, NCT02564523) (Table 2). Moreover, there are 3 phase III studies, adopting the same prime-boost regimen are recruiting subjects or ongoing now. (ClinicalTrials.gov NCT02543567, NCT02509494, NCT02543268) (Table 2).

In addition, there are 6 clinical trials with ChAd3 vaccine as primer and MVA-BN Filo as booster are ongoing (Table 2). In November 2015, the results from one of 6 previous clinical trials (ClinicalTrials.gov NCT02267109) showed a well tolerance and strong immunogenicity of prime-boost regimen.[39] Since administration with MVA-vectored vaccine is capable of extending the duration of protection, more and more studies using MVA-vectored vaccines as booster are been conducted now.

KUN VLPs

Flavivirus Kunjin is an Australian subtype of West Nile virus which is substantially less pathogenic than North American strains of West Nile virus.[42] Reynard et al.[43] developed KUN VLPs expressing Ebola virus GP, which were capable of infecting and delivering replicon RNA into most mammalian cell types. Later the vaccines were evaluated in guinea pig model in 2009, vaccinating female, 3-week-old Dunkin-Hartley guinea pigs with 1×10^{6} or 5×10^{6} vp KUN VLPs expressing full-length wild-type or D637L-mutated GPs or GP/Ctr intraperitoneally.[43] Then, 20 d after the prime vaccination, a boosting immunization with the same dosage and type of KUN VLPs was administrated. Only 25%∼75% of guinea pigs inoculated with KUN VLPs expressing full-length wild-type or D637L-mutated GPs survived after challenging with a lethal dose of 200 LD_{50s} of recombinant guinea pig-adapted EBOV. However, immunization with KUN VLPs expressing GP/Ctr did not elicit any protection.[43] Pyankov et al.[44] injected 4 African green monkeys subcutaneously with 10^{9}vp KUN VLPs encoding GP/D637L per animal twice with an interval of 4 weeks, only 75% animals survived from the challenged 3 weeks later with 600 PFU of EBOV (Table 3). Because of the limited efficacy, the KUN VLPs is far away from applying in humans currently, further studies with higher dosages of the vaccine may need to be investigated.

Replicative vector-based Ebola vaccines

rVSV-EBOV

The rVSV-EBOV vaccine is generated from a live attenuated recombinant, vesicular stomatitis virus (rVSV) encoding the GP of the EBOV Kikwit 1995 strain.[45] The rVSV-EBOV vaccine developed by the Canadian National Microbiology Laboratory was licensed to NewLink Genetics and subsequently sublicensed to Merck, which was responsible for ongoing researches. Early in 2004, a pre-clinical study found that mice were 100% protected from the lethal challenge with 1000 LD_{50} of mouse-adapted Ebola virus after immunization with rVSV-EBOV vaccine.[45] Next year, a complete protection was also proved in monkeys, which were challenged with 1000 PFU of EBOV after inoculating with 10^{7} PFU of rVSV-EBOV.[46] Subsequently, targeted at exploring whether vaccine was suitable for emergency immunization after exposure to Ebola or not, Feldmann et al.[47] immunized animals with the rVSV-EBOV as late as 24 hours after lethal challenge of Ebola virus and achieved 50% and 100% protection in guinea pigs and mice, respectively. More important, 50% of cynomolgus macaques were survived if administrated with rVSV-EBOV 20 to 30 minutes after Ebola virus infection (Table 3).[13] Other preclinical studies also demonstrated a rapid and significant protection in NHPs.[12,14, 48-52]

The first 3 open-label, uncontrolled, phase I clinical trials of rVSV-EBOV vaccine were conducted in Lambaréné, Kilifi, and Hamburg, respectively, which were designed to assess the safety, and immunogenicity of escalating doses ranging from 3×10^{5} to 2×10^{7} vp in early 2014 (NCT02283099, NCT02287480, NCT02296983) (Table 1).[53] The preliminary results of the rVSV-EBOV vaccine from the above 3 trials, involving a total of 99 participants, demonstrated a good immunogenicity, but a mild to moderate reactions related to vaccination (Table 4).

Simultaneously, another double-blind, randomized, placebo-controlled, phase I trial of the rVSV-EBOV vaccine at

dose of 1×10^7 and 5×10^7 vp was conducted in Geneva. A total of 59 participants were administered with one single shot intramuscularly into the deltoid. Of them, 11 (22%) participants without any previous history of joint disease had an onset of arthralgia at a median of 11 d post-vaccination. After magnetic resonance imaging and physical examination, arthritis was confirmed in 9 of 11 participants. The arthritis symptoms in most participants were mild and self-limited with a median duration of 8 d. But at the 6-month visit, there was still one participant who had arthritis with symptoms of swelling on peripheral joints unresolved.[53] Moreover, another 3 participants among the 11 patients with arthritis got a mild maculopapular rash involving fingers or toes, which lasted for 7 to 15 d. It indicated that the dissemination and replication of vesicular stomatitis virus can occur and persist for few weeks after immunization. Because of these unexpected adverse reactions, the study was suspended in December 2014. One month later, this study was resumed with a lower dose of rVSV-EBOV vaccine, which was intended to gain a better tolerance of the vaccine by reducing the dosage, since the preliminary data from another trial in Gabon with a lower vaccine doses suggested a better safety profile of the rVSV-EBOV vaccine but still immunogenic. Therefore, another 56 participants were recruited to continue this trial, with 43 of them were randomly assigned to receive lower dose rVSV-EBOV of 3×10^5 vp or placebo and 13 only received open-label rVSV-EBOV at 3×10^5 vp (NCT02287480).[54] The results showed that the dose reduction to 3×10^5 vp could decreased the occurrence of viremia, but 13 low-dose vaccinees (25%) still occurred arthralgia after immunization. Additionally, another 2 participants reported purpura on the lower legs and the counts of lymphocyte, neutrophil, and platelet decreased significantly at day 1-3 post-vaccination. Although the adverse reactions in those participants received a lower dose rVSV-EBOV at 3×10^5 vp is comparatively mild to moderate with a lower frequency and a similar seropositivity rates observed on day 28 (94%, 48/51) which is very similar with that in previous study participants received high dose at 1×10^7 or 5×10^7 vp, the post-vaccination antibody response level in terms of GMTs were noted significantly lower than high dose (344.5 vs 1064.2). In general, lowing dosage of the rVSV-EBOV vaccine failed to decrease or preclude the occurrence of vaccine-induced arthritis, dermatitis, and cutaneous vasculitis, and moreover, negatively affected antibody responses.

After the reports of arthritis from the above trials, researchers paid more attention to the occurrence of any adverse reactions related to vaccination in another 2 phase I trials with the rVSV-EBOV, which were proceed in the US with a total of 52 adults at the Walter Reed Army Institute of Research (WRAIR) and the NIH Clinical Center[55] (NCT02269423, NCT02280408) (Table 1). But arthritis was neither observed at the WRAIR nor NIH site. The common adverse reactions were injection-site pain, myalgia, fatigue, headache, subjective fever, and chills. Anti-Ebola immune responses were identified in all the participants as well as the VSV viremia with a limit duration. At day 28, GMT of antibodies against EBOV GP were higher in the group receiving 20 million PFU than in the group receiving 3 million PFU (4079 vs. 1300). All these phase I studies with small-scale population facilitated rapid progression to phase II and III trials.

In 2015, a phase III trial was performed in Guinea to assess the efficacy and effectiveness of the rVSV-EBOV vaccine at 2×10^7 vp administered intramuscularly for the prevention of Ebola disease during outbreak.[56] This trial used a cluster randomization design with a ring vaccination approach, which was used for smallpox eradication in the 1970s.[57] A cluster of individuals at high risk of infection defined as contacts or contacts of contacts because of their social or geographical association with the newly confirmed Ebola patient was randomized to receive one dose of rVSV-EBOV vaccine immediately or 21-day delayed. 4123 participants were assigned to immediate vaccination group, and 3528 participants were assigned to delayed vaccination group. No Ebola disease case was found in 4123 participants receiving rVSV-EBOV vaccine immediately, while 16 cases were determined in those received vaccination with 21 day delay, resulting in a complete protection against Ebola disease. Though 43 serious adverse events were reported, only one serious adverse event was judged to be causally related to vaccination, while assessment of serious adverse events is ongoing. The results of this interim analysis are so encouraging that rVSV-EBOV might become the first licensed vaccine in preventing Ebola virus disease. Besides, it also indicated that ring vaccination strategy is most likely effective to target on the population when deliver during an Ebola virus disease outbreak.

Additionally, according to a case report of a physician who was exposed to Ebola virus in treatment unit, he was vaccinated with rVSV-EBOV 43 hours after the exposure.[58] Moderate to severe adverse reactions which are similar to the symptoms of infection of Ebola virus developed 12 hours after vaccination and diminished over 3 to 4 d. Lai et al.[58] detected the blood sample of this patient as showing that Ebola virus GP-specific antibodies and T cells were detectable, but antibodies against Ebola viral matrix protein 40 (not in the vaccine) were not detected. Strong innate and Ebola-specific adaptive immune responses were also detected after vaccination. The clinical syndrome and laboratory evidence were consistent with vaccination response, and no evidence of Ebola virus infection was detected. However, it is unsure that this physician was able to get infected with Ebola virus after having a high-risk occupational exposure without intervention. Besides, it is unknown if rVSV-EBOV is safe or effective for post-exposure vaccination in humans.

Though the most promising rVSV-EBOV vaccine was the first one which demonstrated the great protective effect with highly protection, we still need further observation and studies to identify the efficacy and safety of this vaccine.

HPIV3 vaccine

HPIV3 as a negative-sense RNA virus, which can cause generally respiratory disease among children, has been successfully advanced as a vaccine platform against Ebola virus. In 2005, Bukreyev et al.[59] inserted a transcription casette encoding the GP gene into HPIV3 independently or together with NP gene to formulate this new vaccine. Guinea pigs were protected from challenging with 10^3vp Ebola virus on day 28 after a single intranasal inoculation

of $10^{5.3}$ PFU of experimental vaccine. In rhesus macaques challenging model, 2 doses vaccine were able to achieve a 100% protection while one dose showed only an 88% protection after challenging with 1000 PFU of EBOV[9] (Table 3). Since the impeding factor showed from an epidemiological investigation[60] that PEI to HPIV3 in the human adults may greatly impact the replication and immunogenicity of the vaccine, the vaccine vector was improved by deleting the HPIV3 F and HN genes, which are the main targets for the HPIV3-specific humoral immune response.[61] The new attenuated vector expressing EBOV-GP was more efficient in comparison to the previous construct.[61] One of the main advantages of the HPIV3-based vector platform is the potential for needle-free administration because of this vaccination via the respiratory route. Safety is another virtue of HPIV3, triggered by restriction of virus only in epithelial cells of respiratory tract, an ongoing phase I clinical trial is carried out in US last year and this is one clinical trial which is given intranasally to human beings for now (NCT02564575) (Table 1).

rCMV-based vaccines

rCMV as a novel vector platform was developed because of the unique potential to re-infect and disseminate through target wildlife populations regardless of prior immunity.[62] This vaccine was hypothesized to achieve high vaccine coverage in inaccessible wildlife like apes.[63-65] In 2011, Tsuda et al.[62] constructed a recombinant mouse CMV vector expressing a CD8+ T cell epitope derived from EBOV NP and mice vaccinated with 2 doses were fully protected against 1000 PFU of mouse-adapted EBOV (Table 3). Since as the major advantage of this platform that once the virus has been established in a host, it can continue to replicate autonomously, this type of vaccine is quite relevant for the control of emerging and re-emerging zoonotic infections.[66] This foundation study supported the potential for disseminating rCMV-based vaccines to prevent EBOV transmission in wildlife populations.

rRABV-based vaccines

RABV has been explored as a vaccine platform against Ebola virus recently. For the purpose of reducing neurovirulence to generate Ebola vaccine, Papaneri et al.[67] constructed inactivated and live-attenuated bivalent vaccines expressing EBOV GP based on the SAD B19 strain of RABV. The vaccine candidates were avirulent in adult mice and displayed low neurovirulence in suckling mice. Another study also demonstrated that 5×10^5 FFU of this vaccine could induce humoral immunity and conferred protection from both RABV and EBOV after intraperitoneal challenging with 1000 PFU of mouse-adapted EBOV in mice[68] (Table 3). In 2013, a study immunized NHPs with a platform based on replication competent RABV, replication-deficient RABV, or chemically inactivated RABV expressing EBOV GP.[69] The live replication-competent vaccine provided 100% protection following EBOV challenge while the others provided 50% protection. The results indicated that the protection of immunized animals against EBOV was largely dependent on the quality of humoral immune response against EBOV GP. Hence, utilization of particles containing higher levels of EBOV GP and a boost immunization dose would raise protection rate up to 100% in the animals. Considering of Africa as a high rabies rate region,[70,71] it is significant to produce an effective bivalent RABV/Ebola vaccine as a valuable and remarkable public health tool in that region. However, more safety tests against RABV and EBOV should be conducted before advancing the RABV-based vaccines to clinical trials.

Other Ebola vaccines

Inactivated vaccine

As the first attempting for Ebola vaccine using inactivated virus, it was preformed shortly after the discovery of Ebola virus in 1976. Lupton et al.[72] used formalin or heat-inactivated virus preparations to immunize guinea pigs that challenged with wild-type EBOV later, the results showed that guinea pigs survived with high protection. However, an additional phenomenon should be noticed that only 29% fatality rate of control group was observed in this study, which leaded to doubts about the validity of this study. Later, inactivated vaccine was proved that can provide 100% protection after the challenge with 10 PFU of mouse-adapted Ebola virus in mouse[73] (Table 3). Unfortunately, because of the biological safety hazard and the fact that this inactivated vaccine failed to protect NHPs from challenging of 1000 PFU of EBOV,[41] the continue researches have been suspended in 2002 till now (Fig. 2).

DNA vaccines

Early in 1998, Xu et al.[74] engineered DNA vaccine expressing the GP or NP gene to immunize guinea pigs and the results showed a complete protection. Another study also demonstrated a protection of 100% against challenge with $30LD_{50}$ of mouse-adapted EBOV after immunizing mice with 4 doses of a GP DNA vaccine.[75] Subsequently in 2012, a multi-targeting (trivalent vaccine) DNA vaccine expressing GP genes of EBOV, SUDV and MARV was demonstrated to be highly effective in mice without evidence of interference.[76] Moreover, prime immunization with DNA vaccine and then boosted with adenoviral vectors was shown to protect NHPs from lethal EBOV challenge.[20] DNA vaccine, which induces strong CD4 responses associated with durable immunity, is initially regarded as the most promising and supportive platform against Ebola virus.[77] Since numerous pre-clinical studies have already demonstrated a safety and immunogenicity profiles in animals and the protection related to antibody or cellar response directly[20,21,24] (Table 3), an initial phase I clinical trial with a 3-plasmid DNA vaccine which encoded the envelope GP from EBOV and SUDV subspecies was conducted in 2003.[78] A total of 27 Healthy adult volunteers were enrolled and then arranged into 3 sequential groups to receive placebo or vaccine (5 in the 2-mg dose group, 8 each in the 4-mg and 8-mg dose groups, and 6 in the placebo group respectively). In this study, no serious adverse events were reported, and the experimental vaccine were well-tolerated and safe in healthy adults. Furthermore, Ebola specific humoral responses were successfully detected in all vaccinees and the range of antibody titers was similar to those detected in nonhuman primates.[21] The specific antibodies to each antigen can be induced by the vaccine independently

and are not cross-reactive.[78] However, Ebola virus-specific neutralizing antibody failed to be detected in vaccines as might be expected with DNA vaccination in the absence of boosting with adenoviral vector-based Ebola vaccine.

Moreover, another study published the results of a phase I clinical trial which evaluated the safety, tolerability and immunogenicity of 2 DNA vaccines, one that encodes for MARV Angola GP and the second for EBOV and SUDV wild-type (WT) GP in 2008.[79] (NCT00605514) (Table 1) The first Group was enrolled to receive the MARV DNA vaccine and the second group received the EBOV WT DNA vaccine. Besides, the immunization series was a 3-dose priming regimen with an optional single-dose homologous booster in both groups. After whole vaccination, both the EBOV and MARV WT GP vaccines were well tolerated. The WT GP constructs evaluated in this study were immunogenic and induced both humoral and T-cell responses to all 3 GP immunogen inserts. Also additional administration of a fourth dose of DNA as a homologous boost improved the antibody titers and T-cell responses. This study lighted the evaluation of these 2 vaccine candidates in further clinical trial in Africa for immunogenicity and efficacy.

Then, in 2009, a phase I, double-blinded, randomized, placebo-controlled clinical trial was conducted to examine the safety and immunogenicity of the EBOV and MARV vaccines given individually and concomitantly in Kampala, Uganda[32] (NCT00997607) (Table 1). As the first clinical trial of Ebola virus and MARV vaccines in Africa, 108 participants were enrolled into the study and randomly assigned (in a ratio of 5:1) to receive at least one injection of either vaccine or placebo. In part 1 of the study, participants were randomly assigned to receive EBOV vaccine and MARV vaccine, or placebo. And in part 2 of the study, participants were randomly assigned to receive either EBOV vaccine in the left arm and MARV vaccine in the right arm or a placebo injection in both arms at each of the 3 injection visits. The results showed that, given separately or together, both vaccines were well tolerated and immunogenic to elicit antigen-specific humoral and cellular immune responses (Table 4). All these findings have contributed to the accelerated development of more potential Ebola vaccines that encode the same WT GP antigens.

One of the advantages of DNA vaccine plasmids is that it can be genetically designed to produce proteins from a pathogen with no risk of infection. Additionally, this stable and easily developed vaccine is inexpensive to produce. Considering the broad immunogenicity of this Ebola virus DNA vaccine, immunization by plasmid DNA delivery is a viable platform and merits further development. Up to date, phase III clinical trials of the DNA platform alone or in combination with replication-defective adenoviral vector vaccines are ongoing.[15]

VLPs

Generally, as a new complex protein-based vaccine, VLPs was much safer than inactivated vaccine or attenuated vaccine due to its specific characteristic that without viral genome. It is also capable of stimulating response of both humoral immunity and cellular immunity. Bavari et al.[80] generated Ebola VLP vaccine by the expression of VP40 alone or along with GP and NP in 2002. Warfield et al.[81,82] following demonstrated that mice and guinea pigs immunized with Ebola VLPs and Marburg VLPs respectively, which generated humoral immunity could be totally protected after challenging. In another study performed in 2007, all Ebola VLP-vaccinated NHPs survived 1000 PFU of EBOV challenge after 3 vaccinations. However, an issue that immunization period ups to 6 weeks is too long for achieving emergency needs for rapid immunization in outbreak areas.[83] Besides, it is difficult to produce tremendous amounts of VLPs needed for vaccination because of the expensive, time-consuming and laborintensive production period. For addressing this problem, new approach comes out recently by using the baculovirus expression system to produce VLPs in insect cells, which is proved protection of mice.[84] Furthermore, Warfield et al.[85] adopted the NHPs model to show that one or 2 doses of VLPs vaccine can confer protection from lethal challenge (Table 3). As a vaccine candidate, Ebola VLPs have relatively low biosafety concerns, and their use can bypass issues associated with PEI.

rEBOVΔVP30

rEBOVΔVP30 is a vaccine candidate reported firstly in 2008[86] and generated by using reverse genetics to delete VP30 from the genome of EBOV artificially.[87] Halfmann et al.[86] replaced VP30 from the genome of EBOV with neomycin genome and the virus lose the normal ability of replication and production of progeny without changing of morphology. The team further demonstrated that rEBOVΔVP30 was safe in STAT-1 knockout mice and also evaluated the protective efficacy in mice and guinea pigs which were injected with 2 doses and challenged with mouse-or guinea pig–adapted EBOV subsequently[88] (Table 3). However this vaccine is replication incompetent, its genome still contains more than 95% of the original EBOV genome, some concerns targeted to the safety issue when the generation of viruses reintegrated VP30 into their genome in nature with the horrible consequence. In 2015, Marzi et al.[89] inactivated the vaccine with hydrogen peroxide, the results showed that rEBOVΔVP30 was capable of protecting NHPs against challenging with 1000 PFU of EBOV when given either one or 2 doses. Regardless of the good immunogenicity and efficacy of the rEBOVΔVP30 observed in animal studies, due to the safety concerns of the regaining replication ability, rEBOVΔVP30 was not able to enter the clinical trials for further evaluation.

Conclusions and future directions

In recent 2 years, we have seen a significant accelerated progress in the development of Ebola vaccine, and there were a number of vaccine candidates that performed extremely well through phase I or II clinical trials. Meanwhile some phase III studies with Ebola vaccines are ongoing. Even though the prevalence of Ebola outbreaks had been stopped, and only sporadic cases with Ebola disease had been reported in the past a few months, Ebola virus still attracted tremendous attention worldwide. rVSV-EBOV vaccine is the first vaccine successfully demonstrated a high protective efficacy against Ebola disease in the phase III trial, which may become the first licensed Ebola vaccine. However, there are still several concerns about the rVSV-EBOV vaccine, especially for the safety like immunization

induced viremia and arthralgia. According to previous study results, reducing the dosage of rVSV-EBOV vaccine may not be a useful strategy to improve its safety profile. The adverse reactions associated with the rVSV-EBOV vaccine like fever, myalgia, chill and headache especially post-exposure, needs to be further accessed and distinguished because these reactions are similar to symptoms of infection of Ebola virus apparently. Considering the current clinical trials are mostly conducted in subjects aged over 18 y old and non-pregnant women, we should further design and evaluate the vaccine applied on children, elders and pregnant women. Moreover because of the high rates of positive HIV, rabies or malaria and poorly available treatment care, vaccines should not only be designed for healthy adults, but also for those special groups such as HIV infected, malaria infected or people with other baseline diseases, who were more vulnerable to Ebola.

Besides, the persistence of the vaccine protection and the need of booster injection after prime vaccination to against warning of antibodies still need to be further investigated. Although a single shot may be more efficient for emergency immunization, it may not be enough to induce a durable protection against potent infectious agent, whereas a prime-boost combination can induce broader and durable immunity. Moreover, boosting with the heterologous vaccine could prolong durability of protective immunity and induce stronger immune response to antigens than using homogeneous vaccination, which was shown in different clinical trials and suggested as a prospective approach of vaccination by WHO experts.[30,33,37]

In the past decade, we have witnessed impressive progress in the development of viral vectored vaccine. Recombinant viral vectors are good at delivering heterologous antigens that combine the different favorable features of other vaccine modalities, with minimal disadvantages.[90] Compared to simpler vaccines, viral vectored vaccines which are capable of infecting cells and expressing encoded antigens ensures efficient induction of humoral and cellular responses. Particularly, CD8+ T cells are critical for the elimination of intracellular pathogens, which makes it the key advantage over simpler vaccines. However, the main drawback of viral vector vaccines is that the transgene-specific response may be influenced and dampened. Currently, more and more studies adopted the use of higher doses and heterologous prime–boost regimens to overcome the disadvantage.

So far, the Ebola virus were mostly found circulated in undeveloped areas in Africa. Therefore, an ideal Ebola vaccine should also be highly cost-efficient and affordable. Since the cost of boosting vaccination would be higher than a single-shot immunization, a cost-effectiveness study is also in need.

Furthermore, different vaccination strategies should be considered to achieve a high efficient for controlling the transmission of Ebola virus as post-exposure immunization or emergency immunization in susceptible population in Ebola outbreak areas, like ring vaccination. According to the more and more sporadic flare ups infected by survivors, vaccination strategy should be scheduled in case of this phenomena, we should discuss if there is necessary to use the ring vaccination to get more residents protected.

The 2014-2015 Ebola outbreak was catastrophic in Africa, and it debilitated the local health systems, hampered diagnosis and treatment for endemic diseases like malaria, HIV and tuberculosis and resulted in increasing the mortality rates of other diseases indirectly.[91] Even though a promising vaccine candidate was confirmed, there still remains large gap between production of Ebola vaccines and the vaccination demands. There is a huge vaccine demanding in undeveloped countries, but the poor medical infrastructure will interfere development of vaccine forward. Because the people most at risk for Ebola are the ones least able to pay for vaccines, this leaves little in the way of market incentives for manufacturers to develop vaccines, unless there are large numbers of people who are at risk in wealthy countries. The key reason we still lack of an Ebola vaccine coming to market now is because that there is no market for Ebola. Besides, the vaccine development that taking a well-known antigen and turning it into a viable vaccine is expensive, complicated and long-term process, hence developing an Ebola vaccine is commercially risky and the only reason we have vaccine candidates now is actually because of a somewhat misguided fear.

Moreover, it is meaningful to take standard precautions against Ebola virus like basic hand hygiene, respiratory hygiene, using of personal protective equipment, safe injection practices and safe burial practices. Hopefully, the most promising licensed vaccine will be produced global in the next a few years and also will contribute to world.

Abbreviations

EVD	Ebola virus disease
rVSV-EBOV	recombinant vesicular stomatitis virus-based vaccine
EBOV	Zaire Ebola virus
SUDV	Sudan Ebola virus
BDBV	Bundibugyo Ebola virus
TAFV	Tai Forest Ebola virus
RESTV	Reston Ebola virus
NHPs	non-human primates
WHO	World Health Organization
UK	United Kingdom
US	United States
GP	glycoprotein
VP	virion protein
NP	nucleoprotein
L	RNA-dependent RNA polymerase
MVA	modified vaccinia strain Ankara
VEEV	venezuelan equine encephalitis virus
ChAd3 vaccine	replication-defective recombinant chimpanzee adenovirus type 3-vectored vaccine
ChAd3-EBO-Z	recombinant chimpanzee adenovirus type 3-vectored vaccine expressing wild-type GP from EBOV
cAd3-EBO	recombinant chimpanzee adenovirus type 3-vectored vaccine expressing wild-type GP from EBOV or/and SUDV
KUN VLPs	Kunjin replicon virus-like particle vaccine
vp	viral particles
PFU	plaque-forming units
FFU	focus-forming units

IU	infectious units
HPIV3	human parainfluenza virus type 3
rCMV	recombinant cytomegalovirus
RABV	recombinant rabies virus
VLPs	virus-like particles
rEBOVΔVP30	recombinant EBOVΔVP30
VRP	VEEV replicon particles
rAd5	replication defective human adenovirus 5
NIH	National Institutes of Health
PEI	pre-existing immunity
Ad5-EBOV	recombinant human Ad5 vector based Ebola vaccine
GMT	geometric mean titer
MARV	Marburg virus
MVA-BN Filo	multivalent MVA-BN Filo encoding the GPs from EBOV, SUDV, MARV and a NP from TAFV
WRAIR	Walter Reed Army Institute of Research
WT	wild-type

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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