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A review of Lassa fever vaccine candidates

Kolawole Salami[1], Dimitrios Gouglas[2], Connie Schmaljohn[3], Melanie Saville[4] and Nadia Tornieporth[4]

Lassa fever is a zoonotic disease caused by the Lassa virus, a rodent-borne arenavirus endemic to West Africa. Recent steady increase in reported cases of the disease in Nigeria, where 123 deaths occurred in 546 confirmed cases in 2019 has further underlined the need to accelerate the development of vaccines for preventing the disease. Intensified research and development of Lassa fever medical countermeasures have yielded some vaccine candidates with preclinical scientific plausibility using predominantly novel technology. The more advanced candidates are based on recombinant measles, Vesicular Stomatitis Virus or Mopiea and Lassa virus reassortants expressing Lassa virus antigens, and the deoxyribonucleic acid platform. However, the Lassa fever portfolio still lags behind other neglected tropical diseases', and further investments are needed for continued development and additional research, such as the safety and efficacy of these vaccine candidates in special populations.

Addresses
[1] R & D Blueprint for the Prevention of Epidemics, Room 3170, World Health Organization Headquarters, 20, Avenue Appia, Geneva 1211, Switzerland
[2] Coalition for Epidemic Preparedness Innovations, Marcus Thranes Gate 2, 0473 Oslo, Norway
[3] U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter St, Frederick, MD 21702, USA
[4] Coalition for Epidemic Preparedness Innovations, Gibbs Building, 215 Euston Rd, Bloomsbury, London NW1 2BE, UK

Corresponding author: Saville, Melanie (Melanie.saville@cepi.net)

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Introduction

Lassa fever (LF) is an emerging infectious disease (EID) that has remained a significant cause of morbidity and mortality in the West African sub region since its identification in 1969 [1]. It is caused by Lassa Virus (LASV), an arenavirus that is spread mainly through contact with secretions from infected rodents of the Mastomys species [2]. In 2018, Nigeria experienced the greatest number of LF cases and deaths reported in recent years, with 171 deaths from 633 confirmed cases (An update of Lassa fever outbreak in Nigeria; URL: https://ncdc.gov.ng/diseases/sitreps). There are currently no vaccines or satisfactory drug therapies for the prevention or treatment of LF. Research into the development of these LF medical countermeasures have been slow due to gaps in our understanding of the disease's natural biology and epidemiology as well as limited animal model data. Additionally, development of EID countermeasures has been unappealing for manufacturers, who see little commercial benefit due to the sporadic disease burden and the lengthy, risky, and costly product development process [3]. For survivors, however, immunity is believed to be lifelong, raising the possibility of controlling the disease through immunization [4]. The World Health Organization (WHO) and the Coalition for Epidemic Preparedness Innovations (CEPI) have highlighted LASV vaccine development as a key priority [5]. In June 2017, WHO released the target product profile (TPP) to guide the development of future LASV vaccine candidates [6]. The TPP described the desired qualities of potential LASV vaccines under two circumstances: preventive use (preferable) and reactive/outbreak use. These have led to increased research on the LASV that has produced vaccine candidates with pre-clinical proofs of concept (POC) that might potentially reduce illness, disease outbreaks, and deaths in humans [4]. Here we present a summary of all LASV vaccine candidates that have appeared in the open literature along with a critical appraisal of the scientific plausibility of the platforms and candidate vaccines.

Methodology

An extensive database search was conducted of literature relevant to LASV vaccines between 13 June and 1 September 2018 using PubMed and sources such as reports, conference proceedings, and meeting bulletins. Key search terms in MeSH database included: Lassa fever vaccine 'OR' drug therapy, Lassa fever epidemiology 'OR' transmission, Lassa fever immunology 'OR' microbiology 'OR' virology among others (Table 1).

Results

A total of 1321 articles was obtained, of which 856 articles were selected and manually reviewed. Of these 133 were chosen for inclusion based on date of publication and the nature of article; for example, articles that reviewed existing LASV vaccines candidates or those that reviewed current diagnostic or treatment options in development

Table 1

Lassa fever vaccines in development.

Vaccine technology	LASV antigen	Stage of development	Development partners
rVSVN4CT1-LASV (VesiculoVax™ Vesicular Stomatitis Virus Vector)		Preclinical	Profectus Biosciences; University of Texas Medical Branch
ML29 L-AttV, rLCMV(IGR/S-S) (Mopiea/Lassa reassortant) [14]	GPC, NP	Preclinical	The Scripps Research Institute, USA
VSVΔG/LASVGPC (VSV vector) [24]	GPC	Preclinical	International Aids Vaccines Initiative; Public Health Agency of Canada
RABV-Lassa virus vaccine candidate GPC [26]	GPC	Preclinical	National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH)
YF 17D GPC [27]	GPC	Preclinical	Texas Biomedical Research Institute; University of Louisville; Leiden University Medical Center
ML29 virus – reassortant encodes major immunogenic proteins from LASV and RNA polymerase and Z protein from MOPV. [13]	GPC + NP	Preclinical	Medigen, Inc. (technology licensed from the University of Maryland); NIAID
Live attenuated rLCMV/CD (Based on Codon Deoptimization) [28]		Preclinical	The Scripps Research Institute, University of Rochester, USA
GPC441-449 subunit [22]	GPC	Preclinical	Emergent Biosolutions, University of Vermont, California and The Scripps Research Institute
LASV VLP [18]	GPC, NP, Z Matrix	Preclinical	Tulane University Health Sciences Center; Autoimmune Technologies, LLC; Corgenix Medical Corporation; Vybion, Inc.,; United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
HLA-A02 and 10 HLA-A03-restricted epitopes [22]	GPC	Preclinical	The University of Vermont College of Medicine; University of California; Pharmexa-Epimmune
VaxCelerate subunit [29]	GP1, GP2	Preclinical	Massachusetts General Hospital; EpiVax, Inc.; 21st Century Biochemicals; University of Washington; MPI Research; Pfenex Inc.
RABV based on chemically inactivated rabies virus containing Lassa Virus coGPC (LASSARAB) [26]	GPC	Preclinical	Thomas Jefferson University; NIAID; The Geneva Foundation; USAMRIID; IDT Biologika GmbH; Infectious disease research institute (IDRI)
PODS Lassa 1 [30]	GPC	Preclinical	Cell Guidance Systems; University of Cambridge; Imperial College London; Department of Health - UK
MV-LASV (recombinant measles virus vaccine expressing Lassa virus antigens) [31]	GPC + NP, GPC + Z protein	Preclinical	Institut Pasteur; Themis Bioscience GmbH
MOPEVAC (Modified Mopeia virus expressing antigens of pathogenic arenaviruses) [32]	GPC	Preclinical	Institut Pasteur
Alphavirus replicon encoding LASV genes [33]	GPCwt	Preclinical	Medigen, Inc.; University of Louisville, United States USAMRIID
Lassa GPCclamp (molecular clamp technology) [34]	GPC	Preclinical	The University of Queensland; Australian Government – National Health and Medical Research Council (NHMRC)
ChAdOx1 Lassa [17]		Preclinical	Oxford University
MVA Lassa		Preclinical	Oxford University National Infection Service, Public Health England
MVA Lassa(LassaVac) [35]			
ChAdOx1-biLAMA [17]		Preclinical	Oxford University/Jansen
Viral genome rearrangement for the development of live-attenuated arenavirus vaccines [36]		Preclinical	University of Rochester; The Scripps Research Institute
Single cycle infectious viruses as live attenuated arenavirus vaccines [37]		Preclinical	University of Rochester; The Scripps Research Institute
Digitally designed Immune Optimized and Selected-Lassa, Ebola, Marburg (in silico design of antigen sequences) [38]		Preclinical	University of Cambridge; University of Regensberg; Department of Health-UK
GEO-LM01 [39]		Preclinical	GeoVax; The Scripps Research Institute; University of Maryland
pLASV-GPC is a DNA plasmid vaccine [9]	GPC	First in human	Inovio Pharmaceuticals; USAMRIID
MVA-VLP-TV vaccine (Hemorrhagic Fever Vaccine (Ebola, Sudan, Marburg, Lassa) [40]		Preclinical	GeoVax; USAMRIID

were given preference over articles describing one study only. These 133 articles were reviewed to assess the LASV vaccine pipeline and challenges of developing LASV vaccine candidates. All but one of the vaccine candidates described in our review are currently in the preclinical phase and are mostly based on the Josiah strain of the LASV. Of these, only four have been tested in non-human primates (NHP).

Live-attenuated measles virus (MV)/LASV GPC + NP

Reverse genetic manipulation of the Schwarz strain MV genomes to generate recombinant MV that present antigens of foreign pathogens has been used to develop a live attenuated MV-vectored vaccine expressing LASV GPC + NP (Josiah) [7]. Two vaccine candidates were developed: one expressing LASV GPC + NP and another expressing the LASV GPC and Z proteins. Both have shown promise during assessment in human primary macrophages for their ability to induce type I IFN (IFN-α1, -α2, -β) responses and to induce surface expression of co-activation molecules (CD80, CD86, CD40, CD83). Both also demonstrated robust viral replication and stimulation of innate immune responses essential for mounting efficient adaptive immune responses in animal models of LF [7].

Vesicular Stomatitis Virus (VSV)-vectored vaccine

VSV is a ubiquitous virus that infects animals and human cells. The genomic RNA encodes five major proteins: glycoprotein (G), matrix protein (M), nucleoprotein (N), large protein (L), and phosphoprotein (P). A recombinant VSV has been produced by deleting the original G gene, which effectively removes the cytopathic effect of the virus in the host. Replacement of the VSV G gene with a heterologous glycoprotein gene results in presentation of the foreign glycoprotein on the surface of the attenuated rVSVΔG, yielding a vectored virus combination that has received widespread attention and applicability. It has been used to develop a promising Ebola viral disease (EVD) vaccine candidate (rVSV-EBOV) [8]. Replacement of the VSV G gene with the LASV glycoprotein complex (GPC) gene resulted in the replication competent VSVΔG/LASVGPC with an inability to cause disease, but ability to induce an immune response against LASV. Pre-clinical POC studies of rVSVΔG/LASVGP (Josiah) showed that all NHP vaccinated survived a 10^4 -plaque forming unit (PFU) challenge with LASV Josiah strain 28 days after vaccination, while all animals in the control group developed severe LF and were euthanized. The vaccine induced significant antibody responses measured by ELISA and neutralization assays, in all the 4 intervention NHPs, but only induced a significant cell mediated immune (CMI) response in 3. This suggests that the 4th NHP was likely protected through humoral immunity. This result was replicated in guinea pigs and for other strains of LASV found in Liberia and Sierra Leone. The vaccine appears well tolerated with the only abnormalities noticed being mild blood dyscrasia (thrombocytopenia) and transient elevated alanine aminotransferase (ALT) levels. There was no virus shedding. Transient LASV RNA was however noticed in vaccinated animals, an indication that the vaccine does not induce sterilizing immunity – a highly desirable attribute of a vaccine developed against a supposedly highly contagious disease [9–11].

DNA vaccines

DNA vaccination involves the direct introduction into tissues of a plasmid expressing gene(s) encoding the antigen(s) against which an immune response is sought and relies on the in-situ production of the target antigen. A gene-optimized DNA vaccine that encodes the GPC gene of LASV (Josiah strain) administered in 3 doses delivered by the dermal route followed by electroporation protected guinea pigs from LASV-associated illness and death. The result was replicated in NHPs. Challenge with a lethal dose (LD) of LASV Josiah after 2 or 3 vaccinations delivered by the intradermal route and electroporation 3 or 4 weeks apart resulted in survival and no sign of disease in vaccinated macaques, while all the control macaques developed serious LF disease with 7 out of 10 challenged controls dying. CMI is likely the main protective immune mechanism as low-levels neutralization antibodies were observed [9]. This is the first LF vaccine candidate to commence human trials [10].

Live-reassortment MOPV/LASV (ML29) vaccine

Mopeia virus shares NP and GP2 epitopes with LASV but lacks those epitopes responsible for eliciting full protection against LASV. Researchers have since noticed that genetically closely-related arenaviruses occasionally undergo reassortment during replication in host cells leading to the generation of phenotypically distinct types due to the bi-segmented nature of their RNA. This possibility was further exploited *in vitro* to produce a reassortant clone between LASV and MOPV called ML29 through 18 different mutations in the original viruses. The resulting clone retained the L segment of the non-pathogenic MOPV and the S segment of LASV (Josiah). ML29 is thus an attenuated live virus, but with retention of RNA necessary for the induction of strong immune response. The resultant vaccine was found to be safe, efficacious and immunogenic in all animal models with no noticeable side effect following single dose vaccination. Viral shedding was also rare [11].

The vaccine elicited specific immune responses and protected marmosets against fatal disease by inducing sterilizing CMI [14]. Although it also induced strong (non-neutralizing) antibody responses measurable by ELISA, immunity is believed to be mainly through CMI. Additionally, it was also found to be protective against the Nigerian clades of the LASV [12,13]. The potential for safety and protective efficacy of this vaccine

in HIV-positive populations was suggested by its positive safety and immunogenicity profile in Simian immunodeficiency virus (SIV)-infected macaques [14]. The vaccine also offers the promise of post-exposure prophylaxis as shown by the 100% survival rate in guinea pigs when the vaccine was administered 48 h after infection with an otherwise LD of LASV [15].

In addition to these vaccine candidates, there are a few others that have shown some promise in murine models but their efficacy has not yet been established in more appropriate animal disease models [16].

Live attenuated yellow fever (YF-17D)/LASV

A recombinant YF17D/LASVΔSSP/GPC was produced through genetic engineering of LASV and YFV. Although the vaccine elicited a strong LASV-specific CD8+ T cell protective response in guinea pigs, these results could not be replicated in NHPs. More work is required to enhance immunogenicity and viability of this platform for generating a LASV vaccine candidate [15,16].

Recombinant replication-incompetent rabies virus (RV) vector

Genetic manipulation of the RV gene to express LASV GPC resulted in a vaccine that was found to offer 80% protection against death in vaccinated guinea pigs with high-levels of IgG antibodies being the main immune response. The presence of the IgG correlated with survival despite being non-neutralizing. However, LASV RNA persisted in some of the challenged animals at study endpoint. The vaccine requires further pre-clinical evaluation [17,18].

Chimpanzee adenoviral (ChAd) vectored LF vaccine

ChAd has been found to be safe and a good option to circumvent the problem of pre-existing immunity to human adenoviruses. This platform has been used to develop a vaccine candidate for LF that is immunogenic in mice by the insertion of antigens at the E1 locus of the ChAdOx1 (a species E modified chimpanzee adenovirus based on isolate Y25) through Gateway recombination. The adoption of newer approaches for thermo-stabilization for adenoviral vectors makes this platform especially appealing [18].

Lassa virus-like particles (VLPs) displaying all major antigens

VLPs contain repetitive, high density displays of viral surface proteins that present conformational viral epitopes that can elicit strong T cell and B cell immune response. VLPs have been used to develop vaccines for hepatitis B and human papillomavirus, both of which are FDA licensed, and is currently being exploited for the development of chikungunya and influenza vaccine candidates. A mammalian expression system that generated large quantities of LASV VLPs in human cells has been developed. The resultant VLP contain the major immunological determinants of LASV- the GPC, NP, and Z proteins. Vaccination of laboratory mice with LASV VLPs (3 doses) elicited robust antibody responses in murine models with mature IgG against individual antigens. This platform needs further investigation in the form of LD challenge and POC in suitable animal models [18].

Recombinant vaccinia virus expressing LASV NP and GP

This platform has shown promise of protective efficacy in pre-clinical studies involving guinea pigs and NHPs. Although it appeared the full complement of LASV GPC is essential to survival. CMI is thought to be the main protective immune response since the robust antibody response against LASV nucleoprotein (NP) antigen noticed in animals that received the entire antigens or NP antigen only did not correlate with survival [19]. However, the possible development of immunosuppressive phenotypes of the vaccinia virus through inhibition of human dendritic cells makes it distinctly unappealing. Especially as the vaccine is targeted at sub Saharan Africa, a region with the world's highest HIV/AIDS prevalence. A similar vaccine candidate based on the modified vaccinia Ankara (MVA) virus, a highly attenuated strain of vaccinia virus, is now being evaluated in murine models with initial promising outcomes [20,21].

HLA-A02 and 10 HLA-A03-restricted epitopes

This vaccine candidate is based on an attempt to develop a single vaccine for the 7 pathogenic arenaviruses (LCM, Lassa, Guanarito, Junin, Machupo, Sabia, and Whitewater Arroyo viruses) by targeting an epitope common to them, or a collection of epitopes from them [21]. To evaluate protection, HLA transgenic mice were vaccinated with a cocktail of 14 CD8+ T cell peptides and challenged with a rVACV construct that expressed either LCMV GPC, LASV GPC, or SABV GPC. Protective immunity was observed and was thought to be mediated by a cross-protective CD8+ T cell response. The use of rVACV as surrogate challenge viruses, instead of the virulent arenaviruses (due to lack of BSL 4 facilities) is a major limitation of the study. Further studies involving the use of appropriate challenge strains and animal models that mimic human disease are expected [22].

Discussion

Immunization is one of the most beneficial and cost-effective disease prevention measures. There are global efforts to develop new vaccines for the control of diseases of global health importance. Awareness of LF as a major threat is growing; especially after the 2013–2016 EVD epidemic. However, LF R&D efforts, and indeed all EIDs, still lag when compared to many other neglected tropical diseases with the vaccine pipelines for diseases such as malaria, tuberculosis, diarrheal diseases, salmonellosis and even helminthiasis being more robust than LF's [16].

In recent years, laboratory research on LASV has produced vaccine candidates that are effective in laboratory animals and might potentially drastically reduce illness, disease outbreaks, and deaths in humans. From the 10 WHO Blueprint priority EIDs, Lassa has one of the most advanced (in terms of animal data) and diverse (in terms of volume of candidates and diversity of platforms) pipelines in place today [23,24].

For use in endemic regions, ideal LASV vaccines should be cost-effective and affordable to ensure sustainability. The vaccine should also be stable for a reasonable length of time with no need for extensive cold chain facilities and should confer protection in special populations for example HIV+, pregnant women, and children. Efficacy should be at least 3 years and protection should last at least 3 years with the possibility of an extension with a booster. Its method of delivery should be uncomplicated, and a minimal number of doses should be required to confer long-lasting immunity [6].

Live virus vaccines meet several of these objectives. Notably, the rMV platform was found to offer robust protection in animal models and is planned to be delivered as a single intramuscular injection. Other potential advantages are that the vector is based on a well-established and used viral vaccine, hence clinical development, licensing and field deployment would probably be rapid and straightforward. Also, the familiarity with the vector implies that the characteristics are ideal for up scaling and release at reasonable cost of goods. In addition, because measles vaccines have been in use for years, there have been concerns of the possibility of pre-existing immunity in populations having an anti-vector effect. Results from a phase I clinical trial in Austria in 2014, testing the safety and immunogenicity of a candidate MV-chikungunya vaccine showed this not to be the case for chikungunya [8]. Another promising live virus vaccine platform is rVSV, with at least 2 leading LASV vaccine candidates based on this platform [23,24]. The rapid immune response produced is an indication that the vaccine, much like the MV/LASV vaccine would be useful in both preventive and reactive scenarios. Efforts are ongoing to use this platform to produce multivalent vaccine candidates against LASV, EVD, and Marburg virus.

The DNA platform appears to have desirable properties that conform with the WHO TPP for a LF vaccine. It has the, ability to stimulate both B and T cells, potential stability across a wide range of temperatures and the relative ease of rapid, large-scale manufacture. There was no viral shedding following vaccination of NHPs, hence, little to zero risk of environmental contamination. The technology also circumvents the problem of pre-existing immunity interfering with a 'vector'. The vaccine has the special benefit of being the only LF vaccine with proven efficacy against sensorineural deafness, a major debilitating sequalae of LF [9]. However, this should be interpreted with caution and requires further studies as the alterations of cellular immune responses peculiar to the murine STAT1 −/− challenge model does not necessarily correlate with human disease [25]. The platform has also been successfully used to demonstrate the feasibility of administering more than one DNA vaccine at the same time as vaccinated guinea pigs were protected following challenges with EBOV and LASV [12]. The vaccine, however, probably requires multiple doses to achieve maximal protection. Also, the technology required for delivery (electroporation) introduces additional cost to vaccination and might be a significant challenge to the field deployment of this vaccine in resource-challenged settings like West Africa, where the burden of LF is highest. First in human trials of this candidate vaccine has commenced in the USA [10].

The ML29 live virus vaccine has not only offered protection against LASV in NHPs but has also shown promise of immunogenicity in HIV+ populations, offered sterilizing immunity and post-exposure prophylaxis, which makes it highly promising in both preventive and reactive vaccination regimes. The vaccine has also shown efficacy in pre-clinical studies against LCMV. The vaccine, however, requires more testing in NHPs. The safety concerns with regards to the genetic stability of a recombinant virus needs to be further addressed before progressing to first-in-human (FIH) studies. Considering the maturity of the LF vaccine pipeline, it is expected that most of the advanced vaccine candidates will commence FIH trials in 2019 or 2020. However, there are significant challenges in the clinical testing of these products and eventual delivery to populations in need. Importantly, all vaccines to date are based on the Josiah strain and cross protection with other lineages will need to be generated to ensure a universal LASV vaccine. Lassa fever is endemic in West Africa and efficacy trials are likely possible. However, more data are needed on epidemiology of LF to inform on the feasibility of such trials and clinical trial capacity in LF areas. Also, vaccines clinical development is a capital-intensive project. While funding is guaranteed up to phase II clinical trials for the CEPI-funded vaccine candidates, commitment will be needed for phase III trials.

Conclusion

The LF vaccine landscape now consists of a modestly mature pipeline and a variety of technology approaches to vaccine development that have recently been reinvigorated by the establishment of the WHO Blueprint and CEPI. Most are based on novel technologies rather than the traditional approach to vaccine development. Lassa fever vaccine candidates are moving closer to meeting WHO TPPs on both reactive and preventive use, but time is needed to confirm their usefulness. Most of these vaccines confer protection through CMI, although

evidence for antibody protection is evolving. The immune mechanism of protection in LF needs further research. Reproductive toxicity should be prioritized as part of the development process to kickstart early generation of data that might allow the use of the eventual vaccine candidates in pregnant women.

Conflict of interests

The authors declare no conflict of interest.

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