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Immunology Letters 215 (2019) 1–11

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Review

Vaccine platforms for the prevention of Lassa fever

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ARTICLE INFO

Keywords:
Lassa fever
Lassa virus
Vaccine
T cells
Antibodies
Viral vector

ABSTRACT

Lassa fever is an acute viral haemorrhagic illness caused by Lassa virus (LASV), which is endemic throughout much of West Africa. The virus primarily circulates in the *Mastomys natalensis* reservoir and is transmitted to humans through contact with infectious rodents or their secretions; human-to-human transmission is documented as well. With the exception of Dengue fever, LASV has the highest human impact of any haemorrhagic fever virus. On-going outbreaks in Nigeria have resulted in unprecedented mortality. Consequently, the World Health Organization (WHO) has listed LASV as a high priority pathogen for the development of treatments and prophylactics. Currently, there are no licensed vaccines to protect against LASV infection. Although numerous candidates have demonstrated efficacy in animal models, to date, only a single candidate has advanced to clinical trials. Lassa fever vaccine development efforts have been hindered by the high cost of biocontainment requirements, the absence of established correlates of protection, and uncertainty regarding the extent to which animal models are predictive of vaccine efficacy in humans. This review briefly discusses the epidemiology and biology of LASV infection and highlights recent progress in vaccine development.

1. Introduction

Lassa virus (LASV) was first identified in 1969 after the death of two missionary nurses in Nigeria from an acute viral haemorrhagic fever [1]. The annual incidence of LASV infection ranges from 100,000–300,000 cases, and associated mortality is between 5000 and 10,000 deaths per year [2,3]. Lassa fever is endemic to several West African countries. Sierra-Leone, Liberia, Guinea, and Nigeria are most heavily affected; however, LASV infections have also been detected throughout the Côte d'Ivoire, Togo, Benin, Ghana, Central African Republic, Democratic Republic of Congo, Senegal, and southern Mali [4–7]. As a nonspecific febrile illness with a high proportion of asymptomatic cases, LASV infection is often unreported or misdiagnosed, precluding accurate determination of its true burden.

Over the past three years, documented cases of Lassa fever have been steadily rising in Nigeria, garnering international attention. Laboratory-confirmed cases surged from 106 in 2016 to 633 in 2018 [8,9]. Unsurprisingly, as part of its efforts to establish a global strategy to improve epidemic preparedness and response, the World Health Organization (WHO) has listed LASV as a priority pathogen in need of accelerated research and development for new vaccines, therapeutics, and diagnostics. In this review, we briefly summarise Lassa fever virology, epidemiology, immunobiology, and discuss promising vaccine candidates selected for expedited development.

2. Virology, epidemiology, and pathology

2.1. Viral genome and structure

LASV belongs to the *Arenaviridae* family; it is an enveloped, single-stranded RNA virus with a bisegmented, ambisense genome [1]. The virus is spherical in shape with a diameter ranging between 70 and 150 nm. The envelope surface is smooth with T-shaped glycoprotein spikes, and encloses the genome containing the helical nucleocapsid of 400–1300 nm in length [1]. The viral genome consists of a large (L) and small (S) RNA fragment, 3.4 and 7 kb in size, respectively [10]. The L fragment encodes the RNA-dependent RNA polymerase and a small, zinc-binding (Z) protein [11]. Meanwhile, the S fragment encodes the viral glycoprotein precursor (GPC) and the nucleoprotein (NP) [12,13]. The glycoprotein (GP) spike complex drives host cell entry [14]. The mature form of GP is a trimer of heterodimers, each containing the receptor-binding subunit GP1 and the transmembrane, fusion-mediating subunit GP2 [15]. GPC also encodes a stable signal peptide (SSP) that aids in polyprotein processing into GP1 and GP2, regulation of the pH of infectivity, and provision of chaperone functions during GP maturation [16]. As the only antigen displayed on the viral surface, GP has

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https://doi.org/10.1016/j.imlet.2019.03.008
Received 27 January 2019; Received in revised form 14 March 2019; Accepted 17 March 2019
Available online 23 April 2019

been the focus of recent LASV antiviral and vaccine research. Structural analyses of GP indicate that it is a primary target for neutralising antibody binding [17,18]. However, the role of NP-specific T cells in controlling acute infection and mediating heterosubtypic immunity in animal models and Lassa fever patients, offers some justification for the inclusion of NP in LASV vaccines [19–21].

2.2. Viral ecology

LASV is a zoonotic pathogen, which circulates in rodent reservoirs. For several decades, the natal multimammate rat, *Mastomys natalensis,* was assumed to be the only host of LASV [2]. However, recent evidence indicates that LASV circulates in other rodent species. In fact, the isolation of LASV from the African wood mouse, *Hylomyscus pamfi*, in Nigeria and the Guinea multimammate mouse, *Mastomys erythroleucus*, in Nigeria and Guinea, revealed previously undiscovered LASV strains and the identification of new a phylogenetic lineage [22]. In these rodents, the virus exhibits persistent, asymptomatic infection. Due to their ubiquitous geographic distribution across the West African region, the population-at-risk for Lassa fever is estimated at nearly 60 million people [3,4,7]. LASV fever cases are typically the result of rodent-to-human transmission events, facilitated by contact with infectious rodent urine or faeces in the household environment [2]. This human-vector interface predominantly occurs in resource-limited communities, where access to health care and laboratory diagnostic testing is scarce. Therefore, it is thought that many potential cases of infection are never tested [3]. Human-to-human transmission of the virus is observed, but is most often confined to nosocomial outbreaks [2,3]. In addition to implementing vector-control strategies, vaccination is a key approach to preventing and controlling Lassa fever outbreaks.

2.3. Genetic diversity and 2018 Nigerian outbreak

High degrees of LASV nucleotide polymorphism have been observed, with variation between strains reaching 32% and 25% for the L and S genomic segments, respectively [23,24]. LASV strains are genetically diverse and cluster based on geographic location; however, the precise number of circulating strains is unknown [23,24]. The induction of heterosubtypic immunity against phylogenetically distant strains will be a necessary feature of an effective Lassa fever vaccine.

So far, six distinct clades or lineages have been established. Lineages I-III are localised to Nigeria. Lineage IV circulates in Sierra Leone, Guinea, Liberia, and Côte d'Ivoire [23,24]. The Josiah strain (lineage IV) from Sierra Leone is by far the most well-studied; it is widely used in models of LASV infection and in the design of vaccine immunogens [24]. The emergence of lineage V was elucidated through the phylogenetic analysis of strains from Mali and Côte d'Ivoire [25]. Meanwhile, recently discovered strain Kako, putatively classified as lineage VI, was isolated from *Hylomyscus pamfi* in Nigeria. [22]. Sequencing data from a cluster of imported LASV infections, with the index case originating in Togo, may reveal yet another lineage [26]. There is some evidence to support the occurrence of viral reassortment during multi-strain infection within a single host [24].

During a 2014 outbreak of Lassa fever at tertiary hospitals in Ebonyi and Enugu in southeastern Nigeria, phylogenetic analysis of patient isolates provided strong support for the role of virus transmission between infected individuals [27]. The dramatic increase in cases during the 2018 Nigerian Lassa fever season launched investigations into the possible emergence of a new strain with a higher transmission rate [28,29]. Two sequencing studies, analysing 220 and 120 LASV genomes from infected patients, revealed high variability and derivation from previously circulating viruses rather than from a single dominant strain [29,30]. The data did not indicate phylogenetic clustering of LASV from samples collected at similar time points, which would be expected in the event of increased human-to-human transmission. Therefore, Lassa virus transmission continues to principally occur via independent cross-species transmission events [29,30]. No clear reason for the increase in Lassa fever cases has been found; changes in the distribution of the rodent reservoir population or improved disease diagnosis and surveillance remain possible explanations [30].

2.4. Clinical disease and pathology

The overall case fatality rate for LASV infection is 1–2% [3]. The high degree of seroprevalence of LASV-specific antibodies in the general population residing in the endemic regions, indicates that most infections are mild (or asymptomatic) and do not result in hospitalisation. Symptoms of Lassa fever develop over an incubation period of 7–21 days [31,32]. Lassa fever initially presents as a nonspecific febrile illness, which poses a diagnostic challenge due to the abundance of causative agents of acute fever in West Africa. Vomiting, diarrhoea, chest pain, and headaches are common symptoms [31,32]. In a mild case of LASV infection, these symptoms subside and recovery typically commences 8–10 days after disease onset.

Approximately 15–20% of infections result in moderate-to-severe disease [3]. The case fatality rate is approximately 20% amongst hospitalised patients and increases to greater than 50% in high risk groups, including pregnant women and infants. Severe Lassa fever in pregnant women results in nearly 100% mortality in foetuses [31–33]. High mortality is also observed during outbreaks; in Nigeria 20–30% of confirmed cases of infection have been fatal [9].

In severe cases, patients' condition deteriorates after 6–10 days, resulting in respiratory distress, pleural effusion, haemorrhage, and facial oedema. Increased vascular permeability is considered an indicator of poor disease prognosis. Seizures, shock, and coma have also been documented [31,32]. Fatality due to multiorgan failure generally occurs within two weeks of the onset of symptoms. Histopathological examination of tissues from fatal Lassa fever cases reveals hepatocellular, splenic, and adrenocortical necrosis. Acute renal failure is associated with fatal disease [31,32]. Moderate-to-severe hepatitis is frequently observed in Lassa fever patients, but is not implicated as a primary cause of death [34].

The level of viremia is highly predictive of disease outcome, and typically peaks 4–9 days after the onset of illness. In a study of 137 patients with Lassa fever, mortality risk in patients presenting with viremia above 1×10 [3] median tissue culture infectious dose ($TCID_{50}$)/mL at the time of hospitalisation was nearly four times that for patients with lower viral titres [32]. Nearly all fatal cases exhibited terminal viremia ranging from 1×10 [3 to 1×108 $TCID_{50}$/mL. Survivors clear the virus from their blood about three weeks after the onset of illness [32].

Neurological complications, including sensorineural hearing loss and encephalopathy, are commonly observed in Lassa fever patients [35,36]. Deafness may occur as a result of mild or severe illness and is estimated to effect 25–30% of cases. In approximately half of these individuals, hearing loss is permanent; in the remainder, partial restoration of hearing takes place between one and three months after recovery [35,36]. The potential risk of these immune-mediated adverse neurological effects will be a key consideration for Lassa fever vaccine developers. Greater understanding of the mechanism of LASV infection-mediated hearing loss and the role of vaccine-induced immune responses in this phenomenon will be required before advanced clinical development of vaccine candidates can take place.

There are currently no licensed LASV vaccines. Antiviral treatment of Lassa fever is limited to off-label use of ribavirin, which was reported to reduce the risk of mortality to below 5% if administered within the first six days of illness, in an early study. Benefits are dramatically diminished if the drug is started later in the course of disease [37]. However, further investigation into the efficacy of ribavirin is required, as there is limited recent evidence to support these findings. To this end, two on-going clinical trials (NCT02483260 and NCT00992693) are studying the effects of intravenous administration of ribavirin to

patients with a probable or suspected case of Lassa fever or Crimean Congo Haemorrhagic Fever [38,39].

3. Immunobiology of LASV infection and immune correlates of protection

3.1. LASV infection antagonises the innate immune response

LASV exhibits broad tissue tropism infecting the liver, spleen, adrenal glands, and other organs. The virus primarily targets myeloid lineage cells and antigen-presenting cells (APC), such as dendritic cells (DCs) and macrophages [40,41]. These cell types support high levels of viral replication. LASV infection antagonizes the activation and maturation of APC resulting in impaired antigen processing and presentation. Despite the fact that infected APC migrate to the draining lymph nodes, their maturation remains impeded throughout the course of LASV infection, causing dysregulation of the adaptive immune response and reduced viral clearance [40,41]. The more complete disruption of the function of APC by LASV may correlate with the lack of adaptive immune responses observed in fatal cases. The presentation of LASV antigens by immature DCs may lead to immune tolerance and ultimately immunosuppression [40,41].

Mopeia virus (MOPV), a highly similar, non-pathogenic arenavirus, also infects and replicates in DCs and macrophages at high levels. However, these cells are strongly activated by MOPV infection and increase expression of CD80, CD86, CD40, type I IFN, TNFα, and IL-6 [42]. These observations indicate that high levels of APC activation characterise a protective immune response whereas the impaired activation of these cells, in the case of LASV infection, is a determining feature of pathogenesis [43].

The type I IFN response has been shown to play a critical role in controlling infection during early stages, by suppressing viral replication and contributing to the activation of the virus-specific adaptive immune response [44,45]. Mice deficient for the type I IFN receptor succumb to lethal infection [46]. Additionally, cytokine responses in non-human primates (NHPs) that survive LASV infection indicate strong, transient upregulation of IFNα at early stages of infection. Conversely, upregulation of IFNα expression in non-survivors is only observed at late time points [47].

3.2. T cells play a dominant role in the resolution of LASV infection

As antibody responses remain low during clinical disease, only increasing long after recovery, it is generally thought that resolution of LASV infection is principally mediated by cellular immunity [48]. This assertion is supported by experimental observations of Lassa fever in NHPs; transient but strong activation and proliferation of T cells are observed 5–15 days post-infection in animals that effectively control infection [47]. In contrast, T-cell responses were undetectable in animals developing fatal disease based on the expression of T-cell activation markers, CD69 and CD25, and the proliferation marker, Ki67 [47]. In the NHP model of Lassa fever, severe disease is associated with T-cell depletion in secondary lymphoid tissues, transient lymphopenia, decreased T-cell proliferation, and uncontrolled virus replication [47]. In humans, LASV-specific $CD4^+$ and $CD8^+$ T cells are activated early during infection and continue to be detected after recovery despite a low or absent antibody response [49]. Memory $CD4^+$ T-cell responses against LASV GPC and NP persist for several years after initial infection [49].

Early Lassa fever vaccine development approaches based on γ-irradiated inactivated whole virus did not protect rhesus macaques against lethal challenge, despite the detection of antibody responses against LASV GPC and NP after immunisation [50]. These findings implied a more crucial role for cell-mediated responses in resolving LASV infection. Live-attenuated or viral vector-based vaccines expressing LASV GP and/or NP antigens are favored because they more potently induce $CD8^+$T-cell responses. LASV antigen-specific $CD8^+$ T cells have been detected after vaccination with a live-attenuated MOPV/LASV reassortant (clone ML29), a vesicular-stomatitis virus based vaccine, and an alphavirus replicon particle vaccine in animal models of infection [51–53].

3.3. The role of antibody responses in protection against Lassa fever is poorly understood

LASV-infected individuals produce IgM and IgG antibodies; however, antibodies are produced at relatively low levels during early infection and are not neutralising [48]. Generally, antibody production after LASV infection does not correlate with disease outcome. Neutralising antibodies are detectable months after the resolution of acute infection and titres are initially low [48]. Neutralising antibody titres continue to rise months after convalescence, perhaps due to low levels of persisting virus that continue to stimulate B cells [48]. In seroconverted individuals, antibodies are primarily specific to GP and NP. Structural studies of B-cell antigenic epitopes have identified two binding sites on GP1, six sites on GP2, and four sites on NP [17,54]. The structure of the glycan shield of LASV GP has been implicated as a barrier to the induction of neutralising antibodies during infection, thus potentially limiting the role of B-cell responses in protection [55].

However, the significance of the humoral response in protection may be understated. The administration of human monoclonal antibody therapy in guinea pig and NHP models of Lassa fever has been shown to protect against severe disease [56,57]. The monoclonal antibodies used in these studies had a high neutralising index, potentially suggesting that a strong neutralising response may be protective even though neutralising antibodies are not extensively produced during natural infection [56,57]. Finally, a recent study of an inactivated LASV and rabies virus vaccine has provided evidence for the role of non-neutralising antibodies in virus clearance via antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis in an in vitro model of infection [58]. Therefore, vaccines capable of inducing high titre antibodies may achieve protection despite the fact that antibodies play a lesser role in naturally acquired immunity.

4. Animal models for Lassa fever vaccine development

Given the annual incidence of Lassa fever, vaccine licensure will likely proceed via a traditional approval pathway and multi-site efficacy trials in West Africa, rather than through the US Food and Drug Administration's "Animal Rule" [59]. Moreover, as the molecular pathogenesis of LASV infection and immune correlates of protection are not fully understood, the value of animal models in predicting clinical outcomes to vaccination may be limited. For these reasons, efficient progression of promising vaccine candidates into clinical trial evaluations will be crucial to accurately characterise vaccine-mediated immunity.

Nevertheless, well-defined animal models recapitulating elements of human LASV infection are of critical importance to the compilation of vaccine immunogenicity and efficacy data to inform future clinical trials. Animal models will be also useful in studying vaccine-induced heterosubtypic immunity. Challenge experiments using heterologous strains of LASV would provide the clearest information on cross-protective efficacy; however, representative and reliable lethal models of infection have not been widely established for many different LASV strains. Almost all vaccine efficacy studies have been performed with wildtype or species-adapted Josiah strain LASV (lineage IV). An important area of future research will be the development of susceptible rodent and NHP models of LASV infection for epidemiologically relevant strains, particularly those currently circulating in Nigeria. A more feasible approach to collecting preliminary evidence of heterosubtypic immunity is to conduct antibody and T-cell cross-reactivity assays. Purified antigen and peptide cocktails synthesised for different

strains may be used to detect cross-reactive antibody and T-cell responses in in vitro assays, such as ELISA and ELISpot. Notably, this same approach can be used to assess cross-reactive responses to diverse LASV strains in human samples obtained during future clinical trials.

Many different rodent and NHP species have been explored as models for Lassa fever. A thorough discussion of all Lassa fever animal models under investigation is outside of the scope of this review. Instead, we focus on the most widely adopted models in pre-clinical efficacy studies.

4.1. Rodent models for studying vaccine immunogenicity and efficacy

As a rodent-borne virus, the immune response to LASV infection differs between rodents and NHPs/humans. Nevertheless, certain features of Lassa fever clinical disease and immunobiology can be reproduced in rodents and specific rodent models are widely used to study vaccine-mediated responses during early stages of preclinical development. However, the potentially limited scope of these models to accurately predict vaccination outcomes in NHPs and humans should be acknowledged.

Mouse models offer an economical approach for studying vaccine immunogenicity and obtaining initial efficacy data. Pathogenicity of LASV infection in mice is highly dependent upon the host strain, age, and route of infection. In fully immune competent mouse strains, LASV infection is rarely lethal, particularly when using peripheral routes of inoculation. Antiviral testing and studies of disease pathogenesis have relied on immune knock out strains, such as STAT-1 KO and IFN-α/βR$^{-/-}$ that develop fatal infection, but are not optimally suited for the study of immune responses to vaccination [46,60]. However, intracranial inoculation of young adult inbred CBA/J mice at a dose of 588 LD_{50} has been shown to result in fatal convulsive immunopathological disease resembling lymphochoriomenigitis virus (LCMV) infection. Recent studies have utilized the CBA/J mouse model to evaluate CD8^{+}T-cell responses to multiple vaccine candidates and to assess their role in protection [61,62].

In a recent report, GeoVax Labs, Inc. announced the results of efficacy testing of a Lassa fever vaccine based on their modified vaccinia viral Ankara virus-like particle platform in a mouse model of infection. A single dose conferred 100% protection after lethal LASV challenge [63]. However, this study has not yet been published in peer-reviewed literature and the mouse model used has not been specified.

Inbred strain 13 and outbred Hartley guinea pigs are the most widely adopted small animal models for studying Lassa fever and testing vaccine candidates. Pathogenicity differs based on host strain and virus strain. Intraperitoneal infection with Josiah strain LASV produces a uniformly lethal disease in inbred strain 13 guinea pigs characterised by fever, weight loss, and death within two weeks. Lymphopenia, neutrophilia, and reduced levels of serum albumin are also observed [64,65]. Viraemia is first detectable four days after infection and peaks 10–12 days after infection. High viral titres are found in lymph nodes, salivary glands, spleen, pancreas, and lungs; viral replication is also detected in the liver, heart, brain, kidney, and adrenal glands. LASV infection in guinea pigs may be more myocardiotropic and less hepatotropic than in humans [64,65]. In Hartley guinea pigs, the lethality of Josiah strain LASV infection varies from 30% to over 60%. Viral titres are lower in Hartley guinea pigs and viral replication is limited in comparison with inbred animals [64,65]. Recently, a lethal model of Lassa fever in outbred Hartley guinea pigs was developed after four passages Josiah strain LASV in the host strain [66]. After intraperitoneal inoculation with $1 \times 104TCID_{50}$ of LASV, animals uniformly succumbed to disease by 15 days post-infection. Clinical manifestations of LASV infection resembled those observed in the inbred guinea pig model [66]. The new lethal Hartley guinea pig model is currently being applied to vaccine efficacy studies.

So far, the following LASV strains have been tested in the inbred strain 13 guinea pig challenge model: GA391 (Nigeria, lineage III), Josiah (Sierra Leone, lineage IV), 803213 (Nigeria, lineage II), Z-132 (Liberia, lineage IV), Soromba-R (Mali, lineage V), and Pinneo (Nigeria, lineage I) [20,67,68]. GA391, Josiah, 803213, and Z-132 infections are 100% lethal in all naïve animals within 10–18 days post-infection. Soromba-R and Pinneo infections are not uniformly lethal in the inbred guinea pig model. Naïve animals exhibit signs of disease (i.e. lethargy and weight loss) between 10–12 days post-infection; but some animals subsequently recover while others succumb to disease [68].

4.2. Non-human primate models for assessing vaccine efficacy

Infectious virus challenge of NHPs is considered to most closely replicate human Lassa fever. Lassa fever in rhesus and cynomolgus macaques has been most extensively studied; however, a marmoset challenge model has been applied to vaccine efficacy studies more recently.

Experimental observations of Josiah strain LASV infection in rhesus macaques suggest that viraemia appears 5–10 days post-infection and continues to increase until the animals succumb to disease [69]. Gross pathological changes, including petechiae and mild-to-moderate pleural effusions, are observed in some animals. Virus is detected in many organs, namely adrenal glands, spleen, liver, kidneys, heart, lungs, intestine, pancreas, bone marrow, lymph nodes, thymus, skeletal muscle, salivary glands, ovaries, bladder, brain, cerebrospinal fluid, and ocular fluid. Typically, the largest quantities of virus are found in the spleen, liver, adrenals, bone marrow, and intestines [69]. Similar to human Lassa fever, severe pathology was observed in hepatic, renal, and splenic tissue. However, meningoencephalitis, systemic and pulmonary vasculitis, and skeletal muscle myositis are more pronounced in the rhesus macaque model than in human Lassa fever [69].

The clinical manifestations of Josiah strain LASV infection in cynomolgus macaques include fever, weight loss, depression, and acute respiratory syndrome [70]. Other clinical features include thrombocytopenia, lymphopenia, enlarged spleen and lymph nodes, as well as pathologic alterations in the liver, lungs, and endothelium [70]. These manifestations closely align with observations in humans. Viraemia is detectable 5–10 days after infection and increases until death. High levels of viraemia, elevated liver enzymes, low levels of proinflammatory cytokines (IL-1β, TNF-α, IL-8, IP-10), and depressed T-cell activation are predictors of poor disease outcome [70]. Marked pathology in the liver of infected animals and high IL-6 production correlates with lethal outcome, which has also been observed in humans. The presence of multifocal severe central nervous system lesions is an additional characteristic of terminal illness in cynomolgus macaques [70].

Common marmosets infected with Josiah strain LASV develop systemic disease closely resembling the clinical and pathologic features of fatal disease in macaques and humans [71]. Major symptoms include fever, weight loss, high viraemia and viral RNA loads in tissues, elevated liver enzymes, and terminal illness between days 15 and 20. Histopathological examination reveals multifocal hepatic and adrenal necrosis, interstitial nephritis, and lymphoid depletion [71]. The small size of marmosets relative to macaques may provide a way to reduce the costs associated with NHP efficacy studies and increase ease of handling during experimentation.

There is a paucity of studies on the infection of NHPs with LASV strains from diverse lineages. In addition to Josiah strain LASV, only strain Z-132 has been used in heterologous challenge experiments involving cynomolgus macaques, where it results in uniformly lethal disease between days 12–15 post-infection [68].

5. Accelerated development of vaccines based on platform technologies

Significant progress has been achieved in the preclinical development of Lassa fever vaccines, with numerous candidates demonstrating

efficacy in animal models of infection. The first phase I trial to evaluate the safety and immunogenicity of a Lassa fever vaccine candidate is scheduled to begin shortly [72].

The WHO Target Product Profile (WHO TPP) for Lassa Virus Vaccines provides a guideline for the future comparative assessments of vaccines for deployment in a non-emergency setting. WHO TPP lists a set of minimum and preferred vaccine requirements to prevent Lassa fever among at-risk groups, including healthcare workers and communities in endemic areas [73]. An acceptable vaccine should at least be suitable for use in healthy adults and children, but preferably also safe in infants and pregnant women. Only mild, transient adverse effects related to immunisation will be tolerated and additional data indicating that vaccination does not induce neurological complications associated with Lassa fever (including sensorineural hearing loss and neuropsychiatric side effects) will be advantageous [73]. Demonstration of at least 70% (but preferably over 90%) protective efficacy against infection or disease in clinical trials will be required. However, if trials to measure clinical efficacy are not feasible, animal efficacy data alongside clinical immunogenicity data may be considered instead. While a single-dose regimen will be ideal, up to three-doses conferring protection against LASV lineages I–IV for a minimum of three (but preferably over five) years may be deemed acceptable. Longer term protection should be maintained through booster vaccination. Finally, vaccines must have a minimum shelf life of 12 months at −20 °C and six months at 2–8 °C; however, an optimal candidate would possess a shelf life of at least five years and thermostability at higher temperatures [73].

Although clinical assessments of LASV vaccine candidates will provide the true test of alignment with WHO TPP, early indications may be gathered from preclinical studies and existing clinical data on vaccine platforms previously applied to other infectious targets. It is worth noting, that the current WHO TPP focuses on the requirements of a vaccine for preventive use in a non-emergency setting–rather than in an outbreak scenario–to address the burden of Lassa fever in endemic countries [73]. The desired profile for an outbreak vaccine may differ.

In the following section, we review major platforms employed in past and present Lassa fever vaccine development efforts. Data for vaccine candidates that are discussed in brief are also summarised in Table 1. A more detailed in-text discussion is provided for the candidates that we believe to be the most likely to progress to clinical trials and potentially future licensure: MOPV/LASV reassortant clone 29 and the five vaccines endorsed by the Coalition for Epidemic Preparedness Innovations (CEPI) for accelerated preclinical and early clinical development.

CEPI (https://cepi.net) is a relatively new alliance formed to support and finance vaccine development for the prevention of infectious disease epidemics, for which Lassa fever is listed as a priority pathogen. In the past year, CEPI has awarded contracts to five vaccine candidates based on DNA and recombinant virus platform technologies. Platform-based vaccines are poised for expedited progression to clinical evaluation due to established safety profiles. Each vaccine platform has already been used to produce significant quantities of clinical-grade product, and the ability to manufacture vaccines at scale has been established.

Here, we summarise data from guinea pig and NHP efficacy studies for the aforementioned vaccine modalities. When available, clinical safety and immunogenicity of non-Lassa vaccines for individual platforms will also be discussed.

5.1. Vaccinia virus-vectored vaccines

The use of live-attenuated virus or recombinant viral vectors are an attractive approach to Lassa fever vaccine development. Epidemiological observations of Lassa fever in West Africa suggest surviving a single LASV infection may confer long term protection against fatal disease. Re-infection with different strains further boosts

Table 1
Additional efficacy data for Lassa fever vaccines in Guinea pig and NHP models of infection.

Vaccine	Antigen, strain	Animal model	Dose	Dose no.	Measurement of:		Day of challenge	Challenge strain(s)	Survival	Ref.
					T cells	*Antibodies*				
Vaccinia-Lister viral vector	NP, GA391	Hartley Guinea pigs	1×10^7 PFU	1	—	—	28	GA391	100%	[67]
Vaccinia-NYBH viral vector	GPC or NP, Josiah	Strain 13 Guinea pigs	1×10^7 PFU	1	—	X	28	Josiah	94% NP, 79% GPC	[75]
	GPC, Josiah	Rhesus macaques	1×10^9 PFU	1	—	X	28 to 37	Josiah	100%	[76]
	GPC or GPC + NP, Josiah	Rhesus and cynomolgus macaques	1×10^9 PFU	1 or 2	—	X	62 to 488	Josiah	88% GPC, 90% GPC + NP	[77]
Inactivated LASV	Whole virus, unknown	Rhesus macaques	—	6	—	X	108	—	0%	[50]
Venezuelan equine encephalitis virus replicon	GPC or NP, Josiah	Strain 13 Guinea pigs	1×10^7 IU	3	—	X	84	Josiah	100% GPC, 100% NP	[82]
Yellow fever 17D viral vector	GPC, AV	Strain 13 Guinea pigs	1×10^5 PFU	1	—	X	21	Josiah	80%	[79]
	GP1 + GP2, Josiah	Strain 13 Guinea pigs	5×10^6 PFU	2	—	X	44	Josiah	83%	[80]

immunity. Although it is estimated that up to 18% of Lassa fever cases experience re-infection annually, recurrence of clinical disease has never been documented [74]. Cellular immune responses are implicated as having a major role in resolving LASV infection; live vaccines provide the most effective natural pathway to present antigens to MHC molecules. Moreover, given the potency of live vaccines, they are more likely to meet WHO TPP recommendations by conferring protection after one dose.

Multiple replication-competent vaccinia virus-vectored candidates encoding LASV antigens are featured in Table 1 and have achieved variable efficacy in guinea pig and macaque models of Lassa fever [67,75–77]. Vaccinia-Lister encoding NP from strain GA391 (lineage III) protected 100% of vaccinated outbred Hartley guinea pigs against challenge with strain GA391 LASV [67]. A vaccinia-NYBH-vectored vaccine encoding Josiah strain LASV NP conferred 94% protection against homologous virus challenge in inbred strain 13 guinea pigs. Meanwhile, in the same study, vaccinia-NYBH encoding Josiah strain LASV GPC protected only 79% of immunised guinea pigs [75]. However, when vaccinia-NYBH-GPC was evaluated in a homologous challenge experiment in rhesus macaques, 100% protective efficacy was observed [76]. These studies first indicated that guinea pig models of infection may not be entirely predictive of responses in NHPs. A study comparing vaccinia-NYBH vaccines in inbred strain 13 guinea pigs expressing different regions of Josiah strain LASV GPC and NP, revealed that full length GPC, including both GP1 and GP2 subunits, are necessary for protection against LASV challenge [75]. A follow-up investigation in rhesus and cynomolgus macaques indicated that vaccination with both GPC and NP antigens provided a modest increase (88% versus 90%) in protection compared to vaccination with GPC alone [77].

Despite these promising results, deployment of a replication-competent vaccinia-based vaccine in HIV-1-prevalent populations poses significant safety concerns. Therefore, a LASV vaccine based on this platform is unlikely to be licensed for use in Africa.

5.2. Yellow fever 17D virus-vectored vaccines

The Yellow fever virus 17D (YF17D) platform has been used for the construction of chimeric viruses expressing antigens from a variety of pathogens, including LASV GP. YF17D has an excellent safety and efficacy records. Its use as a vector for Japanese encephalitis, Dengue virus, and West Nile virus vaccines is undergoing testing in phase II and III clinical trials [78]. GPC from LASV strain AV was inserted between YF17D E and NS1 genes. The YF17D/LASV-GPC recombinant virus was replication-competent, deeply attenuated, induced immune responses against both pathogens. Vaccination protected 80% of inbred strain 13 guinea pigs from lethal challenge with Josiah strain LASV [79]. However, production of the recombinant virus was hindered by instability resulting from insertion of full-length GPC into the E-NS1 site. Recombinant YF17D was successfully passaged after reducing transgene size; GP1 and GP2 subunits of LASV GPC were separated into distinct YF17D vectors. Combined vaccination with YF17D/LASV-GP1 and YF17D/LASV-GP2 protected 83% of inbred strain 13 guinea pigs from fatal homologous LASV challenge [80].

Subsequently, the efficacy of YF17D/LASV-GPC prime-boost vaccination was assessed in marmosets after lethal challenge with Josiah strain LASV. The immunisation regimen was not protective and all vaccinated animals died with clinical signs of Lassa fever (Lukashevich IS, unpublished data, reviewed in [81]). These data further support the assertion that responses to immunisation in guinea pig models of infection may not fully predict those in NHPs. Elucidation of the causes of low vaccine immunogenicity will be necessary before YF17D/LASV-GPC is considered as a tenable option to control Lassa fever and Yellow fever in co-endemic areas in Africa.

5.3. Venezuelan equine encephalitis virus replicon particle vaccine

Replication-defective alphavirus replicon particle platforms deliver and transduce encoded antigens while carrying out only a single infection cycle. Thus, they maintain the preferred safety profile of 'killed' vaccines while possessing greater immunogenicity. Immunogenicity studies of RNA replicons derived from Venezuelan equine encephalitis virus (VEEV) show that the vector serves as an adjuvant to promote $CD8^+$ T cells responses to the delivered antigen, which is a particularly attractive feature for LASV vaccine design [53]. VEEV RNA replicons, expressing either GPC or NP of Josiah strain LASV, fully protected inbred strain 13 guinea pigs from lethal infection with Josiah strain LASV [82]. However, three doses of VEEV were required to achieve protection, which while meeting the minimum requirements of WHO TPP, would be far more difficult to deploy in LASV-endemic regions that are rural or limited in their infrastructure than a single-dose vaccine. However, a multi-dose immunisation regimen may be more feasible to administer to certain groups, such as healthcare professionals or military personnel, who would be accessible for follow-up at the designated intervals. Recently, a multivalent VEEV vaccine encoding LASV GPC (LASV VLPV) from distantly related strains, LP (lineage I) and Josiah (lineage IV) was developed [53]. LASV VLPV was protective in inbred CBA/J mice and upregulated $CD11c^+/CD8^+$ dendritic cells were implicated as playing a major role in cross-presentation. Vaccination resulted in induction of cross-reactive multifunctional T-cell responses after stimulation of immune splenocytes with peptide cocktails derived from LASV lineages I—IV [53].

5.4. MOPV-LASV reassortant clone 29

A reassortant vaccine platform, MOPV/LASV (clone ML29) was designed to retain the non-pathogenic profile of MOPV while keeping the desired induction of strong protective immunological responses against LASV. This clone has the genotypic characteristics of the L-segment RNA of MOPV strain An20410 and the S-segment RNA of LASV Josiah strain [20]. A single subcutaneous injection of 1000 PFU of ML29 fully protected inbred strain 13 guinea pigs against lethal challenge with 3,300 LD_{50} of Josiah strain LASV (n = 6) or heterologous challenge with strain 803213 LASV (lineage II, n = 5) delivered via subcutaneous inoculation on day 30 post-immunisation. Vaccinated animals did not express any clinical manifestations of disease or biochemical abnormalities during the observation period (70 days). Histological examinations did not reveal lesions in tissues of ML29-vaccinated animals that were challenged with LASV [20]. Viraemia was not detectable in any vaccinated guinea pigs on day 4, 8, 12, or 21 after challenge, suggesting that ML29 induces sterilizing immunity. Non-neutralising IgG antibodies against LASV, predominantly anti-NP, were detected at the end of the first week of vaccination and peaked shortly after challenge [20].

ML29 was found to be safe, immunogenic and efficacious in marmosets; vaccination was associated with an increase in $CD14^+$ and $CD3^+$ T cells [83]. Subcutaneous ML29 vaccination (1000 PFU) protected marmosets (n = 6) from lethal challenge with Josiah strain LASV (1000 PFU), also delivered subcutaneously. All vaccinated animals survived to the study endpoint (35 dpi) and did not develop clinical signs of infection whereas control animals succumbed to disease. Only one ML-29-immunised marmosets developed transient viraemia; LASV was not detected in the blood or tissues in any other vaccinated animals after challenge [83].

In all tested animal models, replication of ML29 was deeply attenuated, did not induce viremia, or any biochemical or clinical abnormalities. Detailed examination of two rhesus macaques infected with this MOPV/LAS reassortant revealed no histological lesions or disease signs [84]. In response to safety concerns over use of ML29 in HIV-1-prevalent populations, rhesus macaques at advanced stages of simian immunodeficiency virus infection were vaccinated with ML29.

Macaques did not display clinical signs of arenavirus disease nor did they develop chronic infection. All vaccinated animals developed ML29-specific cellular and humoral immune responses [85].

Despite the demonstrated advantages of this platform, as ML29 carries the same risk classification as the vector virus (wildtype MOPV). MOPV is classified in risk group 2 by the EU biosafety regulation and risk group 3 by the US CDC, which poses a significant obstacle for further development of this vaccine.

5.5. DNA vaccine

Inovio Pharmaceuticals' INO-4500 is a DNA vaccine encoding the LASV Josiah strain GPC gene (pLASV-GPC), which is codon-optimised to enhance expression in the recipient species (i.e. guinea pigs or macaques). Strain 13 guinea pigs (n = 8) vaccinated three times via dermal electroporation with 100 μg vaccine, at three-week intervals, were fully protected against intramuscular challenge with a lethal dose of Josiah strain LASV (1000 PFU). Vaccinated guinea pigs remained afebrile with no signs of illness, and did not display viremia up to the study endpoint at 28 days post-infection. By contrast, guinea pigs receiving a mock-vaccination (n = 5) with an empty plasmid or no vaccination (n = 8) developed fevers, displayed signs of illness, lost weight, and succumbed to disease 15–18 days post-infection (dpi). Neutralising antibody titres increased in vaccinated animals and peaked around day 21 post-infection before decreasing gradually by the end of the study. A subset of these pLASV-GPC-vaccinated strain 13 Guinea pigs (n = 4) were re-challenged with 1000 PFU of Josiah strain LASV 120 days after the endpoint of the initial study alongside control animals (n = 4). They were monitored for weight, body temperature, and symptom development up to 30 days post-reinfection. Vaccinated animals survived to study endpoint and never developed signs of disease compared to control animals, which were febrile and succumbed to disease 15–18 dpi [86].

Subsequently, efficacy of three-dose and two-dose vaccine regimens were established in the NHP model. Cynomolgus macaques (n = 10) received two or three 10 mg doses of pLASV-GPC administered (as partial doses of 2.5 mg in four different sites) via dermal electroporation at four-week intervals. Electroporation of pLASV-GPC induced modest levels of neutralising antibodies. NHPs were exposed to 1000 PFU of Josiah strain LASV five weeks after the final vaccination and were monitored for 45 days. No symptoms of illness, fever, or sustained viremia were detected in vaccinated animals after challenge. Most (7 out of 10) mock-vaccinated NHPs succumbed to disease 10–17 dpi, and the remainder became critically ill but persisted to study endpoint, albeit with chronic neurological deficits [87].

INO-4500 is the most advanced-stage Lassa fever vaccine candidate and will be the first to be administered to humans. A phase I trial (NCT03805984) will soon begin recruiting healthy adult volunteers to evaluate the safety, tolerability, and immunogenicity of INO-4500 [72]. This candidate bears the additional advantage of relative ease and speed of manufacture in comparison to recombinant viral vector platforms. Despite these advantages, DNA vaccines exhibit low immunogenicity requiring multiple doses delivered via dermal electroporation in order to enhance vaccine potency and achieve full protection. Widespread adoption of DNA electroporation devices and a multi-dose immunisation regimen will be challenging to implement in rural regions of West Africa where Lassa fever is endemic, particularly during an outbreak. However, as in the case with a VEEV-vectored LASV vaccine, a multi-dose electroporation-delivered DNA vaccine may be administrable to localised groups of priority vaccinees (i.e. to hospital/clinic employees).

5.6. Vesicular stomatitis virus vector platform

Two contracts have been awarded by CEPI to accelerate development of candidates utilizing the recombinant vesicular stomatitis virus (VSV) platform expressing LASV GPC (Josiah strain): International AIDS Vaccine Initiative's rVSVΔG-LASV-GPC and a candidate developed by Profectus BioSciences and Emergent BioSolutions using the VesiculoVax™ vector.

VSV, a negative-sense single-stranded RNA virus from the *Rhabdoviridae* family, has been used to express antigens from a variety of pathogens. Both VSV platforms are replication competent. Clinical trials provide evidence for the safety and immunogenicity of attenuated recombinant VSV vectors in humans. Perhaps most notably, following the 2013–2016 EBOV disease outbreak in West Africa, a VSV vaccine expressing the EBOV GPC (VSVΔG/EBOVGP) demonstrated 100% efficacy in a ring vaccination trial [88]. Protection is determined to be primarily antibody-mediated and to last for up to 14 months in NHP models of infection [89]. Perceived advantages of using the VSV system are its high level of immunogenicity and the fact that there is little pre-existing immunity to the vector that might limit vaccine potency. Limitations of this platform include the absence of a thermostable formula, necessitating unbroken cold-chain for transport and storage. Second, despite replicating to high titres in several cell lines, as a negative-sense RNA virus, rescue of recombinant VSV from plasmid DNA can be cumbersome—requiring co-transfection of five plasmids into a permissive cell line [90,91]. Finally, the reactogenicity of VSV-vectored vaccines may pose a concern; in phase I trials of VSVΔG/EBOVGP, high incidence of fever, headache, chills, fatigue, and myalgia was observed after immunisation [92,93].

In the first study characterizing a VSV-based Lassa fever vaccine (VSVΔG/LASVGPC), cynomolgus macaques (n = 4) were vaccinated intramuscularly with a single dose (2×10^7 PFU) of VSVΔG/LASVGPC and challenged intramuscularly four weeks later with a lethal dose (1×10^4 PFU) of Josiah strain LASV. At the time of challenge, a moderate- or high-level of IgG and low-level neutralising antibody titres against LASV were detected in all vaccinated animals. NHPs vaccinated with VSVΔG/LASVGPC were fully protected from any clinical signs of disease. NHPs receiving a control vaccine (n = 2) began showing signs of illness on day 3; these animals succumbed to disease by 11–13 dpi. By day 7, all NHPs were viremic. VSVΔG/LASVGPC-vaccinated animals cleared the virus by day 10, whereas the control NHPs maintained high viremia until euthanasia [52].

Given the significant genetic variation between strains, further studies tested whether VSVΔG/LASVGPC induced cross-protective immunity against geographically distinct LASV isolates. Strain 13 Guinea pigs (n = 9 per group) received intraperitoneal vaccination with 1×10^6 PFU VSVΔG/LASVGPC and were challenged four weeks later with 1×10^4 $TCID_{50}$ LASV strains from Sierra Leone (Josiah, lineage IV), Liberia (Z-132, lineage IV), Mali (Soromba-R, lineage V), and Nigeria (Pinneo, lineage I) [68,94]. Josiah and Z-132 infections were 100% lethal in all control vaccinated animals within 16–18 days post-challenge. Guinea pigs vaccinated with VSVΔG/LASVGPC showed no signs of illness throughout the experiment and infectious LASV could not be isolated from any tissue samples collected during planned necropsy at 18 dpi. Soromba-R and Pinneo infections were not fully lethal in the Guinea pig model. However, all control vaccinated animals exhibited signs of disease (lethargy and weight loss) around 10 dpi. Some Guinea pigs then progressed to terminal illness, whereas others persisted and recovered after day 22. By contrast, all VSVΔG/LASVGPC-vaccinated Guinea pigs fully resisted challenge and did not display any signs of disease [68].

In the same study, cynomolgus macaques (n = 4) were vaccinated intramuscularly with a single dose (6×10^7 PFU) of VSVΔG/LASVGPC or a control vaccine. Four weeks later, NHPs were challenged intramuscularly with 1×10^4 $TCID_{50}$ of the Liberian isolate (Z-132). Between 5–7 dpi, control NHPs exhibited symptoms of disease, which progressed to terminal illness by day 13. VSVΔG/LASVGPC-vaccinated NHPs did not display any signs of infection and LASV was not detected in blood samples [68]. Sequencing data suggests that LASV strains from lineages II and III are circulating in the on-going Nigerian outbreak;

VSVΔG/LASVGPC has yet to be tested for efficacy against these isolates [29,30]. Nevertheless, these data support the feasibility of effectively protecting against multiple LASV lineages with a single vaccine.

Profectus BioSciences's VesiculoVax platform was developed to improve the safety of the VSV vector. The attenuated vector contains a translocated N gene and a truncated cytoplasmic tail. Immunogenicity of the vaccine has been demonstrated in cynomolgus macaques; mild inflammatory responses were associated with intrathalamic inoculation [95]. This attenuated vector has been used to generate vaccines against EBOV and Marburg virus that are protective in NHPs [96–98]. Data on the VesiculoVax vectored Lassa fever vaccine has yet to be published.

5.7. Measles virus vector platform

Themis Bioscience, in partnership with Institut Pasteur, has developed a replication-competent, live-attenuated Schwarz strain measles virus vector expressing the LASV GPC and NP. The large insert capacity of the vector has enabled stable insertion of both antigens. A single dose of the vaccine, MV-LASV-NP + GPC, protected macaques from lethal LASV challenge (Mateo M, manuscript submitted, reviewed in [99]).

Although not yet demonstrated, a vaccine expressing both LASV GP and NP antigens may induce broad cross-reactivity and a large pool of $CD4^+$ memory T cells against all phylogenetic groups of LASV as T-helper cell epitopes of LASV-exposed individuals carry sequences highly conserved sequences between arenaviruses [21,100]. However, this approach may pose a practical challenge. Many diagnostic tests for Lassa fever are based on detection of NP-specific responses. If NP is also included in a vaccine, then there would be no clear diagnostic marker to distinguish those vaccinated and those infected with LASV.

The safety and immunogenicity of the measles vector platform has been established in human clinical trials of Themis Bioscience's MV-CHIK vaccine to protect against chikungunya virus infection. A randomised-controlled phase 2 trial enrolled 263 healthy volunteers aged 18–55 years to receive MV-CHIK (n = 195), measles prime vaccine and MV-CHIK (n = 34), or a control vaccine (n = 34). MV-CHIK was delivered intramuscularly, at 5×10^4 or 5×10^5 $TCID_{50}$, in two different administration regimens. The primary endpoint was immunogenicity, which was defined as the presence of neutralising antibodies against chikungunya virus. Virus-specific neutralising antibodies were detected in all MV-CHIK-vaccinated groups after one or two doses. No serious adverse events related to the vaccine were reported. Overall, MV − CHIK, was found to be safe, well tolerated, and highly immunogenic in healthy adults. Moreover, pre-existing immunity to the measles vector did not affect the vaccine's immunogenicity. These data support the broader use of this platform for other emerging pathogens, such as Lassa fever [101].

5.8. Replication-deficient chimpanzee adenovirus vector platform

CEPI is partnering with the University of Oxford and Janssen Vaccines & Prevention B.V. to support development of a Lassa fever vaccine comprised of a proprietary chimpanzee adenovirus vector platform, ChAdOx1, expressing the Josiah strain LASV GPC: ChAdOX1-Lassa-GP.

Recombinant adenoviruses have been used in gene therapy and as vaccine vectors for decades [102]. However, the widespread presence of pre-existing neutralising antibodies to common serotypes of human adenoviruses has precluded their immunogenicity and protective efficacy in clinical trials by inhibiting vector-mediated delivery of the antigen [103,104]. Adoption of simian adenoviral vectors, to which humans population are less exposed, has circumvented the problem of anti-vector immunity [105].

The ChAdOx1 vector is derived from chimpanzee adenovirus Y25. It bears the advantage of being replication incompetent due to the deletion of a single transcriptional unit, E1, reducing the risk for adverse effects when administered to humans. Additional deletion of the E3 unit enables insertion of transgenes up to 8 kb in size. Like other ChAd vectors, it achieves persistent, high-level expression of diverse antigens. Modification of the E4 region by substituting open reading frames from human adenovirus-5 optimises replication rate and yield in E1-complemented human cell lines [106].

Despite the lack of adenoviral replication after immunisation, ChAdOx1-Lassa-GP has demonstrated protective efficacy in an outbred Hartley Guinea pig model of lethal (Josiah strain) LASV infection after one dose. GP-specific IgG antibodies were detected after vaccination and were boosted after administration of a second vaccine dose. Additional immunogenicity experiments in outbred CD1 mice provide evidence for the induction of cross-reactive T-cell responses to GP from LASV lineages I-III (manuscript in preparation).

Application of the ChAdOx1 platform to a diverse range of emerging pathogens is supported by multiple studies demonstrating that a single dose of ChAdOx1-vectored vaccine fully protected inbred BALB/c mice, sheep, goats, and cattle from lethal Rift Valley fever, protected 100% of transgenic human dipeptidyl peptidase-4 mice from lethal Middle East respiratory syndrome, and offered inbred BALB/c mice sterilising protection from Zika virus infection [107–110]. Each of these vaccine candidates has potently induced antigen-specific T-cell responses and high titre neutralising antibodies [107–111].

Safety evaluations of ChAdOx1 in humans have been completed for influenza (ChAdOx1 NP + M1) in younger and older adults [112,113]. In a phase I randomised-controlled trial with 49 individuals aged 18–46 years and 24 individuals aged 50 years or above, younger adults received one of four heterologous prime-boost immunisation regimens: ChAdOx1 NP + M1 and MVA-NP + MI given eight weeks apart (n = 12), ChAdOx1 NP + M1 and MVA-NP + MI given 52 weeks apart (n = 12), MVA-NP + M1 and ChAdOx1 NP + M1 given eight weeks apart (n = 13), or MVA-NP + M1 and ChAdOx1 NP + M1 given 52 weeks apart (n = 12). Older adults received either ChAdOx1 NP + M1 alone (n = 12) or ChAdOx1 NP + M1 and MVA-NP + M1 administered eight weeks apart (n = 12). Subjects were monitored for 18 months to record adverse effects and measure T-cell responses. ChAdOx1 NP + M1 was found to be safe and well tolerated. No serious vaccine-related adverse effects were recorded and a lower proportion of older adults experienced adverse effects than younger adults. All immunisation regimens were found to boost pre-existing levels of T cells at all ages. Greater amplification was observed after heterologous prime-boost vaccination than after prime-only vaccination [113].

Safe administration of other ChAd vectors has been documented in children [114–116]. In phase I trials, safety and immunogenicity of heterologous prime-boost vaccination with ChA63 and modified vaccinia virus Ankara (MVA) expressing the multiple epitope thrombospondin adhesion protein (ME-TRAP) malaria antigen were assessed in malaria-exposed children and infants in The Gambia and Burkina Faso [114]. Children (n = 138) were enrolled according to the following age groups: 2–6 years in The Gambia, 5–17 months old in Burkina Faso, 5–12 months old in The Gambia, and 10 weeks old in The Gambia. Within each group, multiple combinations of low or high doses of the viral vectors were tested. The vaccine was well tolerated in all four cohorts of children. Adverse effects were mild in intensity, dose dependent, and resolved quickly. The prime-boost immunisation regimen was found to be highly immunogenic in young children, with the highest levels of antigen-specific T cells measured in 10-week old infants [114].

Controlling infectious disease outbreaks through vaccination requires vaccine stockpiling in at-risk regions, which are often resource-limited and possess low or variable cold-chain capacity. The ability of ChAdOx1-vectored vaccines to be thermostabilised for extended periods of time, without significant loss of potency, makes the application of the platform towards emerging pathogens vaccine development especially advantageous. A ChAdOx1 Rift Valley Fever vaccine (ChAdOx1-GnGc) encoding the viral envelope glycoproteins Gn and Gc was thermostabilised via desiccation on glass fibre membranes in

trehalose and sucrose sugars. ChAdOx1-GnGc was then stored for 6 months at 25, 37, 45, or 55 °C. Immunogenicity of the vaccines was determined by measuring neutralising antibody titres in immunised cattle in comparison to those elicited by a standard cold-chain vaccine. Animals vaccinated with thermostabilised ChAdOx1-GnGc stored for 6 months at 25, 37 or 45 °C mounted a functional antibody response comparable to those receiving the cold-chain vaccine at the same dose; titres were within the range associated with protection against RVF in cattle [117].

Despite the advantages of ChAdOx1 as discussed above, the platform is still in early stages of development. Although efficacy has been demonstrated in animal models, ChAdOx1-based vaccines have yet to complete efficacy assessments in humans. Extended durability of vaccine-mediated immune responses have yet to be evaluated. Furthermore, as ChAdOx1 vaccines are under development for multiple co-endemic infections, including Lassa fever, Ebola virus disease, and *Plasmodium falciparum* malaria, the risk of reduced vaccine efficacy due to anti-vector immunity resulting from previous ChAdOx1 vaccination should be investigated. Careful consideration of these challenges and further study will be required before ChAdOx1-Lassa-GP, or any ChAdOx1-based vaccine candidate, can progress to licensure and deployment. ChAdOx1-Lassa-GP may be useful in both non-emergency and outbreak settings, as it is expected to be effective after a single dose.

6. Concluding remarks and outlook for the future

Lassa fever is one of many diseases concentrated in low- and middle-income countries in urgent need of a licensed vaccine. With the augmented support of entities like CEPI and the WHO, it is likely that multiple Lassa fever vaccine candidates will reach clinical trials within the next few years. In order to feasibly develop, test, manufacture, and distribute vaccines against these diseases, new strategies to reduce development costs must be identified. Use of platforms, like DNA, VSV, MV, and ChAdOx1, with established safety and immunogenicity profiles in humans, will help accelerate early stages of clinical development and decrease manufacturing costs. Whereas opportunities to test the efficacy of novel vaccines against Ebola are few, the number of cases of Lassa fever in West Africa will allow for multiple clinical efficacy trials, which may lead to the licensure and deployment of one or more candidate vaccines. As previously stated, during clinical evaluations, promising vaccine candidates must be identified based on alignment with the WHO TPP.

Until then it will be important to monitor the current situation in Nigeria. Continued efforts to characterise the circulating viruses and to elucidate the cause of the rapid increase in cases are needed. Studies of the vector population and their geographic distribution may also be in order. Finally, it will be important to test the cross-protective efficacy of preclinical vaccine candidates against some of the lineage II and III isolates currently circulating in the Nigerian outbreaks.

In preparation for Lassa fever vaccines to move toward clinical studies, it will be necessary to bolster local diagnostic capabilities with well-validated assays, especially point-of-care or field-based technologies. Improved diagnostic tools require detection capabilities spanning the range of LASV polymorphism and multiplex assays to distinguish Lassa fever from other acute febrile illnesses that are prevalent in the region.

Acknowledgement

Supported in part by contract 276871 awarded by the Coalition for Epidemic Preparedness Innovation, Oslo, Norway. SG is a Jenner Investigator. JP is funded by the National Institutes of Health Oxford-Cambridge Scholarship Program.

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