Pre-clinical development of a vaccine against Lassa fever

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Abstract

Lassa virus (LASV) is a persistent global health threat that causes about half a million cases of Lassa fever each year in Western Africa. Although most cases are mild, the disease can cause significant morbidity and results in as many as 5,000 deaths per year. Since 2015, Nigeria has been experiencing a severe and extended outbreak of Lassa fever, raising concerns that it could spill over into other countries and reach a magnitude similar to the West African Ebola outbreak of 2013–2016. Despite the burden that Lassa fever places on public health, both in Africa and around the world, there are still no clinically-approved therapeutics or vaccines to treat or prevent it. Nevertheless, a number of promising candidate vaccines have been developed over the last several years, and there is a growing political and social determination to drive at least one of these candidates towards licensure.

This paper describes a LASV vaccine candidate that is being developed at Canada's National Microbiology Laboratory. Based on the same live attenuated vesicular stomatitis virus (VSV) vaccine platform that was used to produce the successful Ebola virus vaccine, the VSV-based LASV vaccine has been shown to elicit a potent and protective immune response against LASV. The vaccine shows 100% protection in the "gold-standard" nonhuman primate model of Lassa fever, inducing both humoral and cellular immune responses. Moreover, studies have shown that a single vaccination may offer universal protection against numerous different strains of the virus, and additional studies have shown that immunization with the VSV platform appears to be unaffected by pre-existing immunity to VSV. The next step in the development of the VSV-based LASV vaccine is phase I human clinical trials to assess vaccine safety and dosage.

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Introduction

The 2013–2016 Ebola virus (EBOV) outbreak in Western Africa demonstrated that an outbreak anywhere could pose a threat everywhere (1). With nearly 29,000 cases and over 11,000 deaths, EBOV ravaged the countries of Sierra Leone, Liberia and Guinea, and the disease was ultimately exported to numerous neighbouring countries and some Western nations, including the United States (US) (2). Moreover, the outbreak not only devastated the public health infrastructure of Western Africa, but it also strained the global health response. Thousands of health care workers from around the world were deployed to Western Africa where they suffered a disproportionate burden, and over 50% of those infected with EBOV succumbed to the disease (3). At least part of the reason for the magnitude and severity of this outbreak was the lack of a clinically-approved treatment or a vaccine to prevent it.

In the wake of the EBOV outbreak, as well as the Zika virus outbreak that followed in 2015, the Bill and Melinda Gates Foundation recognized a global need for advanced epidemic preparedness. In collaboration with the Wellcome Trust, the World Economic Forum, and the governments of Norway and India, the Bill and Melinda Gates Foundation co-founded the

Coalition for Epidemic Preparedness and Innovations in 2016. The main objective of this coalition was to fund the development of promising vaccines for emerging pathogens that may cause significant outbreaks in the near future, with the goal of rapid scale-up into phase III clinical trials in the event of an outbreak. One of the pathogens selected for accelerated funding and development by this Coalition was Lassa virus (LASV).

Lassa virus, an enveloped, single-stranded, ribonucleic acid (RNA) virus from the family *Arenaviridae*, is the causative agent of the viral hemorrhagic fever known as Lassa fever. Typically, the virus is transmitted from exposure to the urine or feces of infected *Mastomys* rats, although it may also be spread from human to human through direct contact with infected blood, urine, feces or other bodily secretions. Following an incubation period of one to three weeks, the disease is marked by the gradual onset of fever, malaise, and muscle and joint pain. As the disease progresses, fever and myalgia worsen, and patients may become prostrate. Diarrhea, vomiting and other gastrointestinal disturbances are common, as are retrosternal pain and cough. Hemorrhagic manifestations are uncommon but an indication of a poor prognosis, as is facial edema and pleural effusions.

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Fatal cases culminate in shock and death, whereas nonfatal cases resolve over the course of two to three weeks, during which deafness is common and often permanent (4).

Approximately 300,000 to 500,000 cases of Lassa fever occur in West Africa each year, making it one of the most prevalent viral hemorrhagic fevers in humans (5,6). Although typically only 1–2% of these cases are fatal, the scale of infections pushes the overall number of fatalities up to several thousand per year. Cases of Lassa fever are mainly restricted to the West African countries of Sierra Leone, Liberia, Guinea, and Nigeria; however, imported cases of LASV have been extensively documented, along with human-to-human transmission (7–10). Since 2015, Nigeria has been suffering a prolonged outbreak of Lassa fever, sparking fears of another epidemic that may rival the scope of the recent West African EBOV outbreak. Since the beginning of 2018, LASV has resulted in thousands of suspected cases, 413 confirmed cases, nine probable cases and 114 deaths. Based on confirmed and probable cases, the Nigerian outbreak has had a remarkably high case fatality rate of 25% (11).

Despite the significant burden that LASV places on global public health, the virus remains understudied, with no approved treatment or vaccine. Nevertheless, several candidate LASV vaccines have been identified and await further clinical development, including the LASV vaccine that is under development at Canada's National Microbiology Laboratory (NML). This vaccine is a replication-competent vesicular stomatitis virus (VSV)-based vaccine that has shown remarkable efficacy in LASV disease animal models. In this overview, we discuss the pre-clinical development of the VSV-based LASV vaccine, placing it in the context of the EBOV vaccine that was developed from the same VSV vaccine platform, and we describe some other promising LASV vaccine candidates.

Background

Vesicular stomatitis virus as a vaccine platform

The most effective vaccines against viruses usually use a live attenuated virus. Live attenuated vaccines, such as the measles vaccine, are often more effective at inducing protective immune responses and durable immunity than killed virus vaccines or subunit vaccines. One approach to generating live attenuated vaccines has relied on using a relatively harmless “backbone” virus as a vaccine platform to carry antigens from another, more pathogenic virus. At the NML, we have been working with VSV as a vaccine platform for a variety of different viruses, including EBOV, Marburg virus (MARV) and LASV.

Vesiculoviruses comprise their own genus within the family *Rhabdoviridae* and cause disease primarily in mammals and fish (12). In the Western hemisphere, two vesiculoviruses predominate: vesicular stomatitis Indiana virus; and vesicular stomatitis New Jersey virus (13). Both VSVs are insect-vectored viruses that cause vesicular stomatitis in horses, cattle and swine and cause erosive lesions on the tongue, gums, lips, hooves and teats of infected animals (14). In humans, VSV infection is also possible, although infrequent, and can lead to a self-limiting, influenza-like illness with or without the presentation of vesicular lesions (15–17). Because of the similarity in presentation to foot-and-mouth disease in livestock and agricultural animals,

VSV is considered a reportable disease by the Government of Canada.

The development of a system to engineer *de novo* recombinant VSV from DNA plasmids (18,19) greatly increased the utility of VSV as a vaccine delivery platform (20). Wild type VSV already possessed several qualities that made it a suitable vaccine vector and the ability to engineer recombinant VSV only increased its usefulness. The VSV genome has the capacity to tolerate the addition of large and multiple transgenes, which serve as vaccine antigens (21,22), and the virus itself is capable of replicating to high titers in a variety of cell types (23–25), thus ensuring the ease of vaccine production. Moreover, infection with VSV induces a strong humoral and cellular response (26–28), thereby promoting a robust immune response against the incorporated transgene, and pre-existing immunity in humans is rare (15–17), thereby maximizing the vaccine's effectiveness. Additionally, VSV replicates in the cytoplasm without a DNA intermediate, which precludes the possibility of genetic recombination with the host cell, and the VSV genome is non-segmented, which precludes the possibility of genetic shift. Thus, given the potential for VSV to serve as a safe and effective vaccine vector, it is not surprising that this system has been widely exploited to develop vaccines against numerous viruses, including HIV, for which “first-in-human” evaluations have already been completed (29).

A notable variation of the VSV vaccine platform employed by the NML is known as VSVΔG because it lacks the viral glycoprotein (G), which enables virus entry and serves as the virus's major pathogenicity factor. The removal of VSV G not only attenuates the virus by eliminating its potential to infect the nervous system, but it also provides the opportunity to substitute in an analogous viral glycoprotein, thus directing a potent immune response towards an important antigenic target. This strategy was used to develop the highly successful VSV-based EBOV vaccine, which recently demonstrated 100% efficacy against EBOV disease in a small phase III clinical trial (30). Our work at the NML on the LASV vaccine is built on the same VSVΔG backbone (Figure 1).

Development of vesicular stomatitis virus-based Ebola and Lassa fever vaccines

The development of the VSV-based vaccine against LASV has been closely associated with the development of the VSV-EBOV vaccine. The origins of both vaccines can be traced to a single publication. In 2004, Garbutt and colleagues published the first report of replication-competent VSVs (serotype Indiana; VSIV) expressing the glycoproteins of EBOV, MARV or LASV, referred to as VSV-EBOV, VSV-MARV and VSV-LASV, respectively (31). All three viruses exhibited slightly attenuated growth kinetics compared with wild type VSV, and all three viruses exhibited robust expression of their glycoprotein, as well as the expected proteolytic processing patterns. Moreover, none of these VSVs caused disease in mice, suggesting that they were apathogenic. Garbutt and colleagues then inoculated all three groups of mice with a lethal dose of mouse-adapted EBOV twenty-eight days after their initial inoculation with VSV. All mice developed disease and succumbed—except those that had been originally inoculated with VSV-EBOV. This was the first indication that the VSV-based vaccine platform could be used to elicit an immune response against a heterologous glycoprotein that, in turn, could protect animals from disease.

Figure 1: Vesicular stomatitis virus vaccine platform

(A) A schematic of the genome organization of wild type vesicular stomatitis virus (VSV) is shown on the left, with the open reading frames for the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G) and the RNA-dependent RNA polymerase large protein (L) indicated. A schematic of the VSV virion is shown on the right, with the genome encased within the bullet-shaped virion studded with G. (B) A schematic of the genome organization of VSV-EBOV is shown on the left, with VSV G replaced with the Ebola virus (EBOV) glycoprotein (GP) open reading frame. A schematic of the VSV virion is shown on the right, with the genome encased within the bullet-shaped virion studded with GP. (C) A schematic of the genome organization of VSV-Marburg virus (MARV) is shown on the left, with VSV G replaced with the MARV glycoprotein (GP) open reading frame. A schematic of the VSV virion is shown on the right, with the genome encased within the bullet-shaped virion studded with GP. (D) A schematic of the genome organization of VSV-LASV is shown on the left, with VSV G replaced with the Lassa virus (LASV) glycoprotein precursor (GPC) open reading frame. A schematic of the VSV virion is shown on the right, with the genome encased within the bullet-shaped virion studded with GPC.

Shortly after the efficacy of the VSV-EBOV vaccine was demonstrated in mice (31), the same group published the first characterization of the VSV-LASV vaccine in nonhuman primates (32). Cynomolgus macaques were vaccinated intramuscularly with a single dose of VSV-LASV. The animals neither developed signs of disease nor shed vaccine virus, underscoring the safety of this vector. Twenty-eight days later, the animals were challenged with LASV. All the vaccinated animals survived, and none developed signs of Lassa fever. The vaccine appeared to induce both humoral and cellular immune responses, and there were no marked differences in the blood chemistry or hematology of the animals before and after LASV challenge. In contrast, two control animals vaccinated with VSV-EBOV developed clinical manifestations consistent with Lassa fever and succumbed to disease, with no detectable LASV-specific immune response. This study offered a preliminary—but extremely promising—demonstration of the efficacy of the VSV-LASV vaccine; however, it would be nearly a decade before follow-up experiments were performed.

In 2013, EBOV emerged for the first time in Western Africa and sparked an unprecedented outbreak. The EBOV was now present, and possibly endemic, in the same geographical location as LASV, and concerns were raised that a single vaccine platform may be ineffective if used to vaccinate separately against multiple pathogens. To address this concern, Marzi et al. (33) vaccinated a group of three cynomolgus macaques with a single dose of VSV-LASV and challenged the animals twenty-eight days later with LASV. None of the animals exhibited any signs of disease and the absence of a strong antibody response suggested that vaccination induced sterile or near-sterile immunity. Sixty days later, the same three animals were vaccinated with a single dose of VSV-EBOV and were subsequently challenged with EBOV. Despite high titers of anti-VSV antibodies at the time of the second vaccination, all three animals were completely protected from EBOV infection

and exhibited a robust immune response. Thus, pre-existing immunity to the VSV backbone did not compromise the efficacy of the vaccine, indicating that multiple VSV-based vaccines can likely be used in a single population.

There remained the critical question of whether a single VSV-LASV vaccination could prevent disease caused by multiple LASV isolates, since LASV exhibits a high degree of genetic diversity among geographically separated viruses (34). Safronetz et al. (35) addressed this question first in a guinea pig model of LASV infection, demonstrating that VSV-LASV completely protected animals from three heterologous LASV isolates: Z-132 (from Liberia), Soromba-R (from Mali) and Pinneo (from Nigeria). Likewise, vaccination with VSV-LASV protected cynomolgus macaques from lethal challenge with LASV strain Z-132. These results indicate that a single vaccination may offer universal protection against all strains of LASV and may be deployable over the entire LASV endemic range, which comprises at least nine countries and hundreds of millions of people.

Pre-clinical development of vesicular stomatitis virus-based vaccines

Pre-clinical testing of VSV-LASV in various animal models, including nonhuman primates, has demonstrated that this vaccine is safe and effective at eliciting a broadly protective immune response against LASV, in spite of pre-existing immunity to VSV. Despite the promise of this vaccine, clinical development of VSV-LASV is still pending. Nevertheless, the VSV platform has been extensively tested via the VSV-EBOV vaccine, which has undergone rigorous pre-clinical and clinical development (36,37), including phase III human clinical trials where it demonstrated 100% efficacy (30). Similarly, VSV-MARV has undergone extensive pre-clinical development (38), and VSV-based vaccines have also been developed for other filoviruses, including Sudan and Bundibugyo virus (39,40), all of which show remarkable prophylactic efficacy (36-38). Indeed, work with VSV-based filovirus vaccines over the last several years has contributed significantly to our understanding of filovirus disease and the VSV vaccine platform.

Research on the VSV-EBOV vaccine has identified that the formation of antibodies is a critical correlate of protection (41). Studies with VSV-MARV have indicated that vaccine-induced immunity is durable, remaining effective for at least 14 months in the nonhuman primate model (42). Moreover, the VSV-EBOV vaccine—and, by extension, the VSVΔG backbone—has been demonstrated to be safe in immunocompromised animals (i.e., nonhuman primates infected with simian-human immunodeficiency virus) and livestock animals (43,44). Owing to the poor cross-species protection offered by the monovalent VSV-based vaccines, trivalent and blended monovalent single-dose vaccines have also been developed and demonstrate 100% efficacy, suggesting that the VSV platform can be manipulated and optimized to protect against multiple viruses at once (22,39). Finally, phase I, II and III clinical trials have affirmed the overall safety, tolerability, and immunogenicity of the VSV-EBOV vaccine, even at high doses (25,30,45-49). Of note, rare adverse effects have been observed (30), with one phase I clinical trial recording a relatively high incidence of vaccine-induced arthritis, dermatitis, and vasculitis (45,46).

Assessing the risk to livestock

The use of a live, VSV-based vaccine impacts not only the humans who receive it, but also potentially the animals that come into contact with vaccinated humans. Because VSV is a reportable livestock illness, the use of a VSV-based vaccine carries with it the risk that the VSV vector may impact livestock animals, potentially precipitating an agricultural and regulatory crisis. To address this concern, de Wit et al. (44) inoculated pigs with high doses of VSV-EBOV or wild type VSV and monitored the animals for signs of infection and disease. Remarkably, regardless of the virus used for infection, virus replication was detected in a minority of animals, viremia was absent, virus shedding was minimal, and no animal displayed any overt signs of infection. Given the absence of disease in the pigs following direct inoculation of virus, it is unlikely that a vaccinated human could transmit virus to a pig in a way that would trigger a productive infection with overt signs of disease. Moreover, even in the event of such a transmission, the vaccine virus is unlikely to be maintained in the animal population. This study confirms the safety of VSV-based vaccines and suggests that the potential impact of these vaccines on livestock health is minimal.

Alternatives to vesicular stomatitis virus-based Lassa fever vaccine

In an effort to identify a safe and effective vaccine against LASV, a number of different platforms have been developed over the last several decades (50,51) (Table 1). Replication-competent vaccinia virus-vectored vaccines encoding the LASV nucleoprotein and/or glycoprotein were among the first platforms to be devised and have demonstrated reasonable efficacy in guinea pigs and nonhuman primates (52-56). Due to the immunosuppressive nature of vaccinia virus, further development of this vaccine platform was abandoned out of safety concerns, particularly in immunocompromised individuals (51). The yellow fever virus 17D (YF17D) backbone, which encodes the LASV glycoprotein or glycoprotein subunits, has also been developed as a LASV vaccine, although its immunogenicity is poor and it lacks efficacy in nonhuman primates (50,57,58). Likewise, inactivated LASV failed to protect nonhuman primates from fatal Lassa fever (59). Alphavirus replicons, which are self-replicating RNA molecules expressing foreign antigens instead of alphavirus structural proteins and packaged in virus-like particles, have shown promising results as LASV vaccines, including the ability to promote CD8+ T-cell responses and confer complete protection in guinea pigs, but they await additional characterization (60-62). Notably, a DNA-based LASV vaccine offered complete protection from LASV in guinea pigs and nonhuman primates but required multiple administrations, which may not be practical in LASV-endemic regions (63-65).

In addition to VSV-LASV, the most advanced LASV vaccine candidate is based on a reassortant between LASV and the reportedly non-pathogenic Mopeia virus (MOPV) (50,51). Clone ML29 possesses genetic material from both MOPV and LASV—including the nucleoprotein and glycoprotein genes from the latter—and includes several additional point mutations that are thought to further attenuate the virus (66,70,71). Vaccination with ML29 has been shown to be safe and to elicit a potent and protective immune response against LASV. Indeed, it offers complete protection in guinea pigs and nonhuman primates, remains efficacious when administered up to two

Table 1: Lassa virus vaccine candidates and their evaluation in animal models

Platform	LASV antigen	Guinea pig efficacy	Nonhuman primate efficacy	Reference
Replication-competent vaccines				
Vaccinia virus (Lister)	N	100% survival	-	55
Vaccinia virus (NYBH)	GPC	100% survival	-	54
		-	100% survival (rhesus)	53
		79% survival	-	52
		-	67% survival (cynos) 100% survival (rhesus)	51
	GP1	-	0% survival (cynos)	51
	GP2	-	0% survival (cynos)	51
	GP1 & GP2 (separate vector)	-	100% survival (rhesus)	51
	N	94% survival	0% survival (cynos) 43% survival (rhesus)	51,52
VSV	N & GPC (separate vectors)	58% survival	75% survival (cynos) 100% survival (rhesus)	51,52
	N & GPC (same vector)	-	100% survival (rhesus)	51
	GP1 & GP2 (same vector)	100% survival	100% survival (cynos)	31,32,34
ML29	N	67% survival	-	34
	N & GPC (same vector)	100% survival	100% survival (marmosets)	66,67,68
YFV17D	GPC	80% survival	0% survival (marmosets)	57,69
	GP1 & GP2 (same vector)	83% survival	-	56
Other vaccines				
Inactivated LASV	Inactivated LASV	-	0% survival (rhesus)	58
Alphavirus replicon	N	100% survival	-	60
	GPC	100% survival	-	60
	N & GPC (separate vector)	100% survival	-	60
	GPC & EBOV GP (same vector)	100% survival	-	60
DNA/electroporation	GPC	83–100% survival	100% survival	62–64

Abbreviations: EBOV, Ebola virus; GP, glycoprotein; GP1, glycoprotein 1; GP2, glycoprotein 2; GPC, glycoprotein precursor; LASV, Lassa virus; N, nucleoprotein; NYBH, New York Board of Health; VSV, vesicular stomatitis virus; YF17D, Yellow fever 17D; “-”, not done

days post-infection, is safe in immunocompromised animals and appears to be genetically stable with no propensity to undergo reassortment with pathogenic LASV (67,68,70,72,73); however, until recently, ML29 was classified by the US Centers for Disease Control as a Risk Group 3 pathogen, indicating that further safety validation may be warranted.

Discussion

Lassa virus causes hundreds of thousands of infections each year and results in thousands of deaths (5). Despite the clear threat that LASV poses to public health, the virus, as well as the disease that it causes, remain under studied. Largely for this reason, the World Health Organization has listed LASV as a priority disease in their Research and Development Blueprint that is designed to improve global research coordination, accelerate development of countermeasures and provide a framework for outbreak response (74). This Blueprint aims to develop a five-year accelerated research plan to advance LASV vaccines into phase III clinical trials. Moreover, the Coalition for Epidemic Preparedness and Innovations has committed to funding the advanced development of select candidate LASV vaccines, although exactly which vaccine platforms will be pursued has yet to be announced.

Although significant progress has been made towards the goal of developing a safe and effective LASV vaccine, further research is required. Many important questions concerning the use and efficacy of the VSV-LASV vaccine remain, particularly the question of the mechanism(s) of action. Activated CD8⁺ T-cells were noted in a majority of nonhuman primates vaccinated with VSV-LASV (32), suggesting that the cellular immune response plays an important role in protection. Indeed, control of Lassa fever in nonhuman primates has been correlated with the circulation of activated CD4⁺ and CD8⁺ T-cells (75), and nonfatal Lassa fever in humans has been shown to be associated with high levels of T-cell-attracting chemokines (76-78). Conversely, the humoral response to LASV infection does not appear to play a significant role in recovery from infection (75,79-81), and neutralizing antibodies seem to be poorly elicited (32,69,75,78). In contrast to the VSV-EBOV vaccine, in which antibodies play a critical role in protection (41), the humoral response appears to play little role in the protection elicited by VSV-LASV vaccine, although more work is required in this area. Time-to-immunity, immune durability and post-exposure therapeutic efficacy of the vaccine also remain to be investigated. Finally, whether the VSV-LASV vaccine, like the EBOV vaccine, is safe and efficacious in immunocompromised individuals is of particular concern should the vaccine ever be deployed in LASV endemic regions, where HIV-1 seropositivity is high. Despite the work that remains to be done, VSV-LASV is still among the most promising of the LASV vaccines currently in development.

Conclusion

The VSV-LASV vaccine is ready for further clinical development. A panel of experts surveyed by *Science* magazine has already identified it as one of two LASV vaccine candidates with the most potential (82). Not only does the VSV-LASV vaccine offer complete protection against a number of different LASV strains, but the platform upon which it is built, VSVΔG, has also been extensively characterized. Although safety concerns have been

raised regarding the VSV platform, particularly in the context of the EBOV vaccine, the majority of available clinical trial data suggests that VSV-EBOV is both safe and effective. Likewise, the vector appears unlikely to pose a threat to livestock animals.

The next step in the development of the VSV-LASV vaccine is phase I human clinical trials to assess vaccine safety and dosage. As LASV continues to exact its perennial toll upon Western Africa, including the outbreak currently affecting Nigeria, the political and social will to develop a safe and effective vaccine against this disease has never been stronger. The VSV-LASV seems well positioned to be part of the solution to reduce the threat that LASV poses to the world.

Authors' statement

LB – Writing – original draft; writing – reviewing and editing
DRS – Writing – original draft; writing – reviewing and editing
XQ – Writing – reviewing and editing; supervision
DS – Initial conception; writing – reviewing and editing; supervision
LB and DRS contributed equally to this article.

Conflict of interest

The authors declare no conflict of interest.

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