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LETTER

Recombinant vesicular stomatitis virus vector vaccines for WHO blueprint priority pathogens

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ABSTRACT

The devastating Ebola virus (EBOV) outbreak in West Africa in 2013–2016 has flagged the need for the timely development of vaccines for high-threat pathogens. To be better prepared for new epidemics, the WHO has compiled a list of priority pathogens that are likely to cause future outbreaks and for which R&D efforts are, therefore, paramount (R&D Blueprint: https://www.who.int/blueprint/priority-diseases/en/). To this end, the detailed characterization of vaccine platforms is needed. The vesicular stomatitis virus (VSV) has been established as a robust vaccine vector backbone for infectious diseases for well over a decade. The recent clinical trials testing the vaccine candidate VSV-EBOV against EBOV disease now have added a substantial amount of clinical data and suggest VSV to be an ideal vaccine vector candidate for outbreak pathogens. In this review, we discuss insights gained from the clinical VSV-EBOV vaccine trials as well as from animal studies investigating vaccine candidates for Blueprint pathogens.

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Introduction

Emerging infectious diseases are a major threat to public health. Ebola virus (EBOV) and other high-threat pathogens have recently been declared one of the top ten health threats of 2019 by the World Health Organization (WHO).[1] Out of the five Public Health Emergencies of International Concern (PHEIC) that WHO has declared to date, four have been caused by emerging or re-emerging viruses. The last few years, humanity experienced novel and increasingly frequent outbreaks for which timely and efficient countermeasures were lacking. Between 2013 and 2016, more than 11,000 people lost their lives to the dramatic West African Ebola virus disease epidemic. In 2015, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) demonstrated to the world how rapidly a virus can spread into new regions due to travel, when a 68-year-old Korean man returning from the Middle East was infected by MERS-CoV and became the index case for a chain of secondary mainly nosocomial transmissions cumulating in 138 MERS-CoV infections, including 36 deaths.[2] The 2016 Zika virus outbreak in the Americas and its resulting cluster of associated neurological disorders and neonatal malformations,[3] also was declared a PHEIC, to name just a few recent outbreaks that emphasize the urgency to create strategies to contain future outbreaks fast and effectively. In the future, socio-economic factors including an increased global human population and lifespan, urbanization, intensified global travel and mobility and the effects of climate change will further ripen conditions for the emergence and rapid spread of viruses between and among populations. Vaccines are the most effective way to prevent and control this viral spread; however, few vaccine candidates for emerging infections exist. As a result of this phenomenon of supply vacuum, novel viral outbreaks such as the EBOV crisis found the global medical community ill-prepared, and the deployment of a preventive vaccine was delayed due to the lack of clinical-stage vaccine candidates.

In response, WHO initiated a Blueprint list of priority diseases to accelerate Research & Development efforts for pathogens that bear a particularly high potential to cause epidemics while no or limited specific countermeasures are available and may, therefore, cause future public health emergencies.[4] WHO's R&D blueprint, composed and regularly updated by an international committee of experts, identifies and defines priority pathogens on the basis of a number of criteria weighted by their relative importance. Major prioritization criteria focus on whether and how the pathogen is transmitted to humans, the extent of medical countermeasures available, and the severity and case-fatality rate of the corresponding disease. Other factors such as potential societal impacts and the evolutionary potential of the pathogen are also taken into account.[5] The current Blueprint, reviewed in February 2018, identified as present priority diseases Crimean-Congo Hemorrhagic Fever (CCHF), Ebola Viral Disease (EVD) and Marburg Viral Disease (MVD), Lassa Fever, MERS and Severe Acute Respiratory Syndrome (SARS), Nipah and henipaviral diseases, Rift Valley Fever (RVF), Zika disease as well as "disease X", a – yet unknown – disease.[6]

In this context, the detailed characterization and assessment of vaccine platforms capable of being applied to swiftly develop vaccines against a variety of pathogens are of utmost importance. The ideal vaccine candidate for outbreak

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scenarios should be able to be manufactured in a rapid and scalable fashion, be safe, induce both strong cellular and humoral immune responses and rapidly confer long-term immunity after a single immunization. Viral vector vaccines can express a variety of heterologous antigens and, therefore, represent ideal vaccine platforms for developing novel vaccine candidates. As such, vaccine candidates based on the *Rhabdovirdae* family have shown to feature many of these properties.

Vesicular stomatitis virus (VSV) is a negative-stranded RNA virus and a member of the *Rhabdoviridae* family, consisting of a simple genome organization encoding five structural proteins: the nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G) and the RNA-dependent RNA polymerase (L).[7] During the last decade, the recombinant VSV (rVSV) platform has been tested for multiple emerging and neglected viral diseases as well as for therapeutic cancer vaccines in animal studies.[8]

Based on the successful establishment of a method to generate VSV from DNA by Rose et al., effective VSV cloning strategies have been implemented[9] and multiple studies revealed the capacity of VSV to express foreign antigens and thus its potential to be used as vaccine candidates.[10–13] Benefits of the rVSV platform include (reviewed in[14,15]):

(i) fast production of high titers and its propagation in almost all mammalian cells;
(ii) lack of reassortment and corresponding potential to undergo genetic shift *in vivo*;
(iii) inability of the vector's viral RNA to integrate into the host genome;
(iv) simple genetic modification with the possibility to accommodate one or multiple antigenic inserts;
(v) the low seroprevalence in the human population;
(vi) mild pathogenicity in humans;
(vii) induction of humoral and cellular immune responses.

A commonly used VSV vaccine design strategy utilizes an rVSV vector lacking VSV-G – rVSVΔG – which is modified to encode the glycoprotein (GP) of the pathogen of interest in replacement of VSV-G, expressing the foreign GP on the viral membrane.[15] Besides being an efficient and stable way to insert antigens of interest, this approach holds two major advantages. It serves as an attenuation factor, as the pathogenicity of wild-type VSV has largely been attributed to VSV-G. An exchange of glycoproteins may affect cell-tropism and, therefore, additionally, attenuate the vaccine candidate.[14,16,17] Toxicity and pathogenicity are thereby significantly decreased leading to a more benign safety profile. Simultaneously, antibody responses to wild-type VSV are mostly directed against VSV-G in humans and, therefore, eliminating VSV-G is an efficient way to significantly reduce preexisting vector-specific immunity.[18] It, however, needs to be considered that rVSV is a replication competent vector and that vulnerable populations including immunocompromised individuals, pregnant women, infants and the elderly may be at higher risk for adverse events.

The present review now endeavors to summarize pre-clinical and clinical data of VSV vaccine candidates against emerging infectious diseases. We here specifically focus on those viruses listed by WHO as prioritized pathogens in the R&D Blueprint, and for which VSV vector vaccine candidates have been described: Ebola, Marburg, Lassa, Nipah, Zika, and CCHF viruses as well as MERS- and SARS-coronaviruses. We place a particular emphasis on the Ebola vaccine candidate VSV-EBOV, as this vaccine is currently the most advanced candidate of the rVSV platform and the only one that has entered human clinical trials besides an rVSV-vectored HIV vaccine candidate[19] and an oncolytic vaccine (NCT02923466, both in phase I).

Ebola virus

Ebola virus (EBOV), in the genus of *Ebolavirus* and the family of *Filoviridae*, is one of the six *ebolavirus* species, Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV, previously Côte d'Ivoire ebolavirus), Reston ebolavirus (RESTV), Bundibugyo ebolavirus (BDBV),[20] and the newly discovered Bombali virus (BOMV).[21] EBOV is the etiological agent of EVD, which can result in hemorrhagic fever and multi-organ failure. The high case-fatality rate and potential threat posed by EBOV not only as an emerging virus but also as a potential bioterrorism agent, led the first research efforts on vaccine candidates against EBOV to be initiated more than a decade ago. However, when the West African EVD epidemic was declared a PHEIC by WHO on August 8th, 2014,[22] swift development of a preventive vaccine became an absolute priority. In light of this development, today's most clinically advanced rVSV-vectored vaccine candidate – and the most advanced EBOV vaccine candidate to date – has entered clinical trials at an unprecedented pace. The vaccine has been known under multiple names, such as rVSVΔG-ZEBOV-GP, rVSV-ZEBOV, VSV-EBOV, and V920. For reason of uniformity, VSV-EBOV is used to reference this vaccine throughout this review.

VSV-EBOV is derived from a chimeric VSV Indiana strain, in which VSV G is replaced by the transmembrane GP of Ebola virus (formerly Zaire ebolavirus (ZEBOV), now designated EBOV),[20] derived from the Kikwit strain. VSV-EBOV was first described by a group led by Heinz Feldmann and Ute Stroeher at the National Microbiology Laboratory of Canada's Public Health agency. While the objective of the study was to assess EBOV GP pathogenicity in mice using VSV as a vector to express EBOV GP, the researchers found that inoculated mice did not develop EVD, and did, in fact, not show any symptoms after challenge with a mouse-adapted EBOV strain.[17,23] In a following non-human primate (NHP) study, vaccinated animals were completely protected from homologous challenge,[24] and subsequent studies in NHPs provided further evidence for the tolerability and efficacy of the vaccine, administered via several routes of administration – orally, intranasally or intramuscularly, and also when receiving a high-dose aerosol EBOV challenge.[25,26] Furthermore, in mice, even doses of as low as two plaque-forming units resulted in protection.[27] To date, VSV-EBOV has been described to be immunogenic and to elicit high titers of binding and neutralizing antibodies over a wide dose range

in small and large animal models as well as in humans (reviewed in Monath et al.).[16]

In 2016, the results of phase I clinical trials generated by the VEBCON (VSV-EBOV consortium with four independent, yet harmonized investigator-initiated trials in Switzerland, Germany, Gabon and Kenya) provided a first safety and immunogenicity assessment of VSV-EBOV in humans.[28] Results of four more phase I trials in the United States and Canada were reported simultaneously (Regules et al., NEJM 2016) or shortly thereafter.[29–31] To date, a total of 17 phase I-III clinical trials have been completed or are ongoing (summarized in Tables 1 and 2).[16,32] VSV-EBOV is furthermore the only vaccine that is being deployed during the 10th EBOV outbreak in the Democratic Republic of the Congo (DRC). This outbreak now constitutes the worst EBOV outbreak in the history of the country and the second-worst outbreak globally, and has consequently been recognized as a PHEIC on July 17th, 2019. The recent developments emphasize the importance of a detailed evaluation of the ample pre-clinical, clinical and field data on VSV-EBOV gathered to date in order to ultimately expedite licensure and facilitate vaccine supply.

Clinical dose-finding and vaccination strategy

In clinical trials, various dose levels have been assessed, ranging from 3×10^3 plaque-forming units (PFU) to 1×10^8 PFU.[32] Considering both risk and benefit profiles evaluated in human dose-finding phase I trials, a dose of 2×10^7 PFU was determined as the preferred dose[30] and was consecutively used in phase II and III trials[33–37] as well as in WHO's vaccination strategy during subsequent EBOV outbreaks. Due to the high demand for vaccine doses in the growing DRC outbreak and, consecutively, to a relative vaccine shortage, WHO's Strategic Advisory Group of Experts (SAGE) on Immunization has now, however, revised this recommendation for the current outbreak. Potency data from the currently deployed vaccine lots show that a 2-fold dosage reduction would on average still approximate a concentration of 2×10^7 PFU, and, therefore, a 50% reduced vaccine dose is recommended to be given to high-risk contacts (including health-care workers as well as contacts and contacts of confirmed EVD cases). In addition, a vaccine with a 5-fold dose reduction is recommended to be utilized more widely (i.e. in broader populations in which EVD cases occurred), potentially preventing tertiary EVD cases more rapidly.[38] Efficacy data for this dosage, however, need to yet be generated.

In all clinical trials, VSV-EBOV was administered intramuscularly and primarily as a single-shot regimen – a regimen that had previously been shown to be protective soon after administration in NHP.[15,24–26] These tested single-shot regimens are indispensable in outbreak situations, especially in volatile regions with insufficient infrastructure where follow-up vaccinations are often not possible. Yet, in comparison, prime-boost strategies utilizing VSV-EBOV may be beneficial when durability of the vaccine response needs to be expanded. First data have been gathered in a phase I trial, where a subgroup of 30 individuals received a prime-boost regimen (days 0 and 28) of VSV-EBOV at three different dose levels. The vaccinees developed higher antibody (Ab) and virus neutralization titers compared to participants who only received a single-shot; yet these observations were no longer statistically significant six months after initial vaccination.[29] Prime-boost regimens are further evaluated in ongoing studies: The PREVAC study is presently assessing a prime only as well as a prime-boost regimen (at days 0 and 56) of VSV-EBOV in comparison to other vaccine strategies in an estimated 4500 volunteers, including children from 1 year of age, with a follow-up time of 12 months. Another ongoing study, the PREPARE study investigates immune response durability and vaccine efficacy is investigated in persons at occupational risk of EBOV exposure, such as health-care workers and laboratory personnel, across a three-year timeframe. Here, participants are either vaccinated with a single shot of VSV-EBOV or receive a booster immunization 18 months after initial vaccination. In addition, participants whose antibody levels decrease beneath a predefined threshold will also be offered a booster vaccination.

Safety data

As studies published to date differ widely across variables such as size, study population characteristics, dosage, etc., a comprehensive concluding safety assessment has not been made. Nonetheless, VSV-EBOV has been described as safe and overall well tolerated, while also being reactogenic in the clinical studies published to date. The adverse events documented were generally mild to moderate and transient. Importantly, there were no vaccine-related serious adverse events (SAE), other than one case of fever and two allergic reactions (one of them when the vaccine was co-administered with amoxicillin) – all which were resolved without sequelae – in now over 18,000 individuals who have received the vaccine in clinical trials.[16,33] Besides local reactogenicity, which was reported in up to 90–100% of the volunteers,[28,29] the most frequently reported adverse events were systemic solicited reactions such as headaches, which were reported in 21–71%, fever in 10–50%, and fatigue in 12–50% of vaccinees in all published phase II/III studies.[33-37] An initially unexpected adverse event constituted the emergence of arthritis cases that tested PCR-positive for rVSV in the phase I trial in Geneva, Switzerland. The study was halted when 11/51 volunteers developed oligoarthritis after having received 1×10^7 PFU or 5×10^7 PFU of VSV-EBOV and was ultimately resumed with a reduced dose of 3×10^5 PFU. Arthritis occurred on median 11 days after vaccination and lasted for 8 days. The development of arthritis did, however, not correlate with the dosage, as 13/51 individuals who had received the reduced dose likewise developed arthritis. In the reduced dose cohort, the risk of arthritis furthermore correlated with increasing age.[39] It was also observed that its development correlated with higher vaccine efficacy.[40] Three affected participants also developed rVSV-PCR positive skin lesions, with infectious rVSV recovered from one participant. After the study halt, simultaneously running phase I studies of VSV-EBOV included arthralgia/arthritis as a solicited adverse event, but while transient arthralgia was likewise reported, the high frequency of arthritis cases was not observed to the same extent.[28–31,41] Finally, arthritis frequency was assessed in a large placebo-controlled phase III trial. Here, the incidence of arthritis or joint swelling was 4.9% in a study population of 1050 individuals. Its onset and duration was comparable to the observations in the Geneva trial, and again, age was associated with a higher

Table 1. VSV-based EBOV vaccine clinical phase I trials.

Title (ClinicalTrials.gov/PACTR)	Study Acronym	Study ID	Phase	Sponsor	Study Site Location	Number of Particpant Enrollment	Status	Study Focus and distinguishing Feature	Reference
Vaccine Treatment for Ebola Virus in Healthy Adults (V920-001)	V920-001	NCT02269423	I	Merck Sharp & Dohme Corp., USA	USA	39 (actual)	Completed (August 2015)	Dose finding: 3×10^6, 2×10^7, 1×10^8 PFU, placebo.	Regules et al., NEJM 2017
Safety and Immunogenicity of Prime-Boost Vesicular Stomatitis Virus (VSV) Ebola Vaccine in Healthy Adults (V920-002)	V920-002	NCT02280408	I	Merck Sharp & Dohme Corp., USA	USA	39 (actual)	Completed (December 2015)	Dose finding: 3×10^6, 2×10^7, 1×10^8 PFU, placebo. Assessment of prime-boost regimen (days 0,28). Follow-up 1 year.	Regules et al., NEJM 2017
Phase I Trial to Assess the Safety, Tolerability and Immunogenicity of a Ebola Virus Vaccine (VSVΔG-ZEBOV)	V920-003	NCT02374385	I	Dalhousie University, Canada	Canada	40 (actual)	Completed (June 2015)	Dose finding: 1×10^5, 5×10^5, 3×10^6 vs placebo.	Elsherif et al., CMAJ 2017
Phase I Trial to Assess the Safety, Tolerability and Immunogenicity of a Ebola Virus Vaccine (rVSVΔG-ZEBOV-GP)	V920-006	NCT02283099	I	Universtiy Medical Center Hamburg-Eppendorf, Germany	Germany	30 (actual)	Completed (November 2015)	Dose finding (3×10^6, 2×10^7 or 3×10^5 PFU) in healthy adults.	Agnandji et al., NEJM 2016
A Study to Find Out if the New Ebola Vaccine is Safe and Stimulates Immunity That Might Protect Adults in Kilifi, Kenya.	V920-008	NCT02296983	I	University of Oxford, UK	Kenya	40 (actual)	Completed (results published April 28, 2016)	Dose finding: 3×10^6 or 1×10^7 PFU.	Agnandji et al., NEJM 2016
Trial to Evaluate Safety and Immunogenicity of an Ebola Zaire Vaccine in Healthy Adults	rVSV-EBOV-01	NCT02718469	I	Profectus BioSciences, Inc., USA	USA	38 (acutal)	Completed (September 15, 2016)	Safety assessment of novel vector rVSVN4CT (rVSVN4CT1-EBOVGP1) in healthy adults (18-60 yrs). Dose finding (2.5×10^4, 2.5×10^5, or 2×10^6PFU). Prime-boost (day 1 and 28) regimen. Randomized, double-blind, placebo-controlled trial.	
Placebo Controlled, Dose Response, Safety and Immunogenicity Study of Vesicular Stomatitis Virus (VSV) Ebola Vaccine in Healthy Adults (V920-004)	V920-004	NCT02314923	I	Merck Sharp & Dohme Corp., USA	USA	512 (acutal)	Completed (June 23, 2016)	Testing of multiple dosages: 3×10^3, 3×10^4, 3×10^5, 3×10^6, 9×10^6, 2×10^7, 1×10^8 PFU vs placebo.	Heppner Lancet ID 2017
A Phase 1, Open-Label, Dose-Escalation Study to Evaluate the Safety and Immunogenicity of the BPSC1001 (rVSVΔG-ZEBOV-GP) Ebola Virus Vaccine Candidate.	V920-007	PACTR201411000919191	I	University Hospital Tuebingen, Germany	Gabon	155 (actual)	Completed (results published October 6, 2017)	Includes safety and immunogenicity data in children (6 years and above).	Agnandji et al., Plos Med 2017
VSV-ZEBOV Geneva Vaccine Trial (VSV-ZEBOV)	V920-005	NCT02287480	I/II	Geneva University Hospital	Switzerland	115 (actual)	Completed (January 2016)	Dose escalation: 1×10^7, 5×10^7.	Agnandji et al., NEJM 2016

All trials except for "rVSV-EBOV-01" are utilizing the vaccine candidate VSV-EBOV.
All vaccinations are given intramuscular at a dose of 2x10^7 PFU and as a single-shot, unless otherwise noted. V920 trial numbers adapted from Monath et al.
PACTR = Pan African Clinical Trial Registry.

Table 2. VSV-based EBOV vaccine clinical phase II, III and observational trials.

Title (ClinicalTrials.gov/PACTR)	Study Acronym	Study ID	Phase	Sponsor	Study Site Location	Number of Particpant Enrollment	Status	Study Focus and distinguishing Feature	Reference
Partnership for Research on Ebola Vaccines in Liberia (PREVAIL)	PREVAIL, V920009	NCT02344407	II	NIAID, USA	Liberia	1500 (actual)	Ongoing (June 1, 2020)	Included a small HIV positive cohort.	Kennedy et al. NEJM 2017
Immunogenicity of Recombinant Vesicular Stomatitis Vaccine for Ebola-Zaire (rVSV[Delta]G-ZEBOV-GP) for Pre-Exposure Prophylaxis (PREP) in People at Potential Occupational Risk for Ebola Virus Exposure	PREPARE, V920013	NCT02788227	II	NIAID	USA, Canada	40 (actual enrolment: March 25th, 2019)	Ongoing (October 31, 2021)	Durability of immune responses over 3 years, evaluation of vaccine boost in subgroup after 18 months Study population: Health care/frontline workers	
African-Canadian Study of HIV-Infected Adults and a Vaccine for Ebola - ACHIV-Ebola	ACHIV-Ebola, V920-015	NCT03031912	II	Dalhousie University, Canada	Canada	200 (estimated)	Ongoing (December 2019)	Evaluation of safety and immunogenicity of VSV-EBOV in HIV-infected individuals, 13-65 years (prevention)	
Partnership for Research on Ebola VACcinations (PREVAC)	PREVAC, V920016	NCT02876328	II	NIAID	Guinea, Liberia, Mali, Sierra Leone	4500 (estimated)	Recruiting (March 2019, final data collection date for primary outcome measure)	Comparison of preventive vaccine regimes in an African population (healthy, 1 year and older): Ad26.ZEBOV +/- boost with MVA-BN-Filo; VSV-EBOV +/- VSV-EBOV boost, placebo. Focus on immunogenicity.	
STRIVE (Sierra Leone Trial to Introduce a Vaccine Against Ebola) (STRIVE)	STRIVE V920011	NCT02378753	II/III	CDC, USA	Sierra Leone	8651 (acutal)	Completed (December 5, 2016)	Randomized, unblinded trial, immediate vs. deferred treatment of health care workers/ frontline personnel. Safety assessment. No efficacy outcome as no EVD cases occured during study duration. Reports safety in participants who became pregnant after having received the vaccine.	Samai et al., JID 2016
Ebola Vaccine Ring Vaccination Trial in Guinea, Part A	Ebola Ça Suffit!, V920-010	PACTR201503001057193	III	WHO	Guinea, Sierra Leone	5837 (acutal)	Completed (results published December 22, 2016)	First efficacy trial, includes children from 6 years of age.	Henao-Restrepo et al., Lancet 2017
Ebola Vaccine Ring Vaccination Trial in Guinea, Part B	V920-018	PACTR201503001057193	III	WHO	Guinea	2115 (actual)	Completed (results published September 25, 2018)	Includes safety data from participants who became pregnant after having received the vaccine.	Juan-Giner et al., Vaccine 2018
Evaluation of the Safety and Immunogenicity of Three Consistency Lots and a High-Dose Lot of rVSV-ZEBOV-GP (V920 Ebola Vaccine) in Healthy Adults (V920-012)	V920-012	NCT02503202	III	Merck Sharp & Dohme Corp., USA	USA, Canada, Spain	1197 (actual)	Completed (September 29, 2017)	Safety assessment of three lots of 2×10^7 PFU and a high dose of 1×10^8 PFU	Halperin et al., JID 2017
Providing Additional Information on the Safety and Effectiveness of an Ebola Vaccine	V920-01	NCT03161366	IIIb	MSF, Netherlands	The Democratic Republic of the Congo (DRC), Uganda	500 (actual)	Completed (November 30, 2018)	Performed during current EBOV outbreak in the DRC, ring vaccination strategy, individuals 1 year and older.	
PREVAIL VI: Identification of Host Genetic Factors Underlying Ebola Virus Disease Risk, Mortality, Long-term Sequelae, Viral RNA Persistence, Humoral Immunity, and Ebola Vaccine Response	PREVAIL VI	NCT03098862	Observational	NIAID	Liberia	9000 (estimated)	Recruiting (April 30, 2022)	Genetic variation associated with anti-EBOV IgG magnitude in vaccine recipients and EVD survivors 3 years and older	

(*Continued*)

Table 2. (Continued).

Title (ClinicalTrials.gov/PACTR)	Study Acronym	Study ID	Phase	Sponsor	Study Site Location	Number of Particpant Enrollment	Status	Study Focus and distinguishing Feature	Reference
Persistence of the Immune Response After Immunisation With Ebola Virus Vaccines (PRISM)	PRISM	NCT03140774	Observational	University of Oxford, UK	United Kingdom	169 (estimated)	Ongoing (July 31, 2020)	Long-term (5 year) humoral and cellular immunogenicity after primary vaccination with distinct vaccines.	
Immune Durability After VSV-EBOV Vaccination		NCT02933931	Observational	Geneva University Hospital, Switzerland	Switzerland	100 (estimated)	Active (January 2020)	5 year immunogenicity assessment after vaccination with distinct doses in the Geneva study (Agnandji et al., NEJM 2016)	Huttner et al., Lancet ID 2018

Notes:
All trials except for "rVSV-EBOV-01" are utilizing the vaccine candidate VSV-EBOV
All vaccinations are given intramuscular at a dose of 2x10^7 PFU and as a single-shot, unless otherwise noted.
V920 trial numbers adapted from Monath et al.
NIAID = National Institute of Allergy and Infectious Diseases

incidence, which was more pronounced in the lower dose cohort (here: 2×10^7 PFU vs. 1×10^8 PFU).[35] The first emerging data from a small subset of 90 surveyed individuals vaccinated during the current EVD outbreak in the DRC seem to mirror the safety assessment obtained in these clinical trials, and while adverse events were frequent, arthralgia and rash were reported in only 7% and 5%, respectively.[42] Importantly, vaccine satisfaction was reported to be high, which is a very encouraging finding, as misconceptions about the vaccine and EVD constitute a serious challenge for health-care workers to administer vaccinations and care in the current outbreak setting.

Besides the assessment of adverse events, early clinical studies also focused on evaluating potential viral shedding by vaccinated individuals via testing of saliva and urine. In the NHP model, viral shedding was previously not observed.[24] In humans, rVSV viremia could be detected early after vaccination with resolution 3–5 days p.v.; however, viral shedding was extremely rare and presented in individuals with very high viremia.[31] Notably, children (6–12 years of age) and, less pronouncedly, adolescents were found to have higher viremia and shedding frequency than adults when vaccinated with 2×10^7 PFU of VSV-EBOV, raising the question whether lower vaccine doses might be required in pediatric populations.[41]

Vaccine assessment in vulnerable populations

It is important to note that vaccine safety was primarily assessed in healthy adults. Pregnant women and children are, however, disproportionally affected by EVD and safety data from these populations are scarce but essential.

Until now, a small number of children have been included in clinical trials. Despite the observed aforementioned higher viremia in a total of 40 pediatric individuals by Agnandji and colleagues, the vaccine had a comparable safety and immunogenicity profile to that of adults receiving the same dose.[41] A subsequent phase III trial as well as vaccine usage under expanded access showed an acceptable safety profile in over 500 vaccinated children aged 6–17.[33,43] Unpublished and ongoing trials are now also including children aged 1 year and above (Médecins Sans Frontières (MSF) (NCT03161366) and PREVAC trials) and will hopefully provide more data on safety and efficacy of VSV-EBOV in the pediatric population.

To date, pregnant women have been excluded from VSV-EBOV vaccine trials due to the safety concerns of administering a live-attenuated vaccine to this vulnerable population. At the same time, mortality rates are exorbitantly high with a lethality of around 90% in EVD-affected pregnant women and roughly 100% for the unborn children. Of those pregnant women who have inadvertently received the vaccine, no case outcomes have been published to date. However, 43 women reported pregnancies within 2 months of receiving the vaccine in a controlled phase II/III trial. Reassuringly, in this cohort pregnancy losses did not occur more often than in the control cohort and no congenital anomalies were observed.[37] The policy that pregnant women are not being offered the possibility of receiving VSV-EBOV has been a topic of repeated debate and controversy.[44,45] MSF among others, have strongly argued in favor of inclusion of pregnant women, given the enormously high probability of death once infected. In view of the above, the Congolese Ministry of Health, backed by WHO, has recently announced

a change of this policy, offering pregnant and lactating women as well as children under the age of 12 months who are contacts of identified EVD cases the vaccine as of June 15th [46,47] which is not only vital for the protection of this population, but is also an opportunity to generate data on vaccine safety and efficacy in this group and will be of great value in future outbreaks.

As EVD outbreaks occur in a population with high HIV incidence rates, it is all the more important to evaluate how safe the vaccine is in immunocompromised individuals. VSV-EBOV was reported to be tolerable in severely immunocompromised NOD-SCID mice[48] and was consecutively tested in a simian-human immunodeficiency (SHIV) model. SHIV infected macaques did not develop overt symptoms such as fever after vaccination with 1×10^7 PFU of VSV-EBOV, a dosage previously shown to be protective in NHPs. The immunogenicity of the vaccine was limited, and EBOV-GP-specific antibodies could not be detected prior to challenge. When challenged with a lethal dose of EBOV 31 days post-vaccination (p.v.), 4/6 of vaccinated animals showed clinical signs of illness and two of them ultimately died. These two animals had been most affected by SHIV as they displayed the highest SHIV viremia and had the lowest CD4+ T cell counts in the group. Also, unlike the survivors, they did not show VSV viremia after vaccination.[49] This study underlines the need for a further assessment of VSV-EBOV in immunocompromised individuals and probably for adapting vaccine dosage and schedule. One phase II trial of VSV-EBOV to date has included a small subgroup of 22 HIV-positive individuals: no SAE was reported in HIV-positive participants within one month of vaccination, but immunogenicity as measured by antibody responses was found to be lower as compared to HIV-negative individuals.[34] To generate data in a larger cohort, the ongoing ACHIV phase II trial is specifically examining the safety and immunogenicity of VSV-EBOV in an estimated 200 HIV-positive individuals.

Efficacy of VSV-EBOV and utilization during outbreaks

VSV-EBOV contains the GP of the EBOV Kikwit strain, yet, in an NHP model, it showed cross-protection against the heterologous West African outbreak strain of EBOV Makona.[50] The subsequent phase III trial "Ebola Ça Suffit" is the only completed clinical trial reporting human efficacy data of VSV-EBOV to date – and, as such, is the only trial reporting efficacy data of any EVD vaccine at the time of this review. The study was conducted towards the end of the West African epidemic and has yielded highly encouraging results. The trial utilized a ring-vaccination strategy: a cluster of contacts and contacts-of-contacts around a case of EVD was identified and randomized to receive either immediate or delayed vaccination after 21 days, the duration of one incubation period reported for EBOV, and EVD cases from day 10 p.v. were assessed.[33,51] As the study reported a vaccine efficacy of 100%, randomization was lifted and all ring contacts were offered the vaccine, now also including children. None of the 3796 immediately vaccinated individuals developed EVD from 10 days post vaccination (p.v.), while EVD occurred in 16/2041 contacts of the delayed vaccination cohort plus in seven never vaccinated contacts. The perfect efficacy rate had initially been questioned and spurred a controversial debate since the trial design was unblinded and, as the number of EVD cases had already decreased, only few EVD cases were reported in the delayed vaccination cohort. The authors', however, rejected the notion that trial results were biased due to the open trial design.[52,53]

On the basis of these efficacy data, VSV-EBOV has received Priority Medicine (PRIME) designation by the European Medical Association (EMA) as well as Breakthrough Therapy designation by the Food and Drug Administration (FDA), which enables a faster licensure process. The submission of a rolling licensure application to the FDA has been initiated by the manufacturer Merck in November 2018.[54] Owing to its PRIME and Breakthrough Therapy designation, VSV-EBOV has been pre-approved for emergency use during outbreaks by SAGE as part of its Expanded Access framework (also called compassionate use program), while pending licensure.[55] Under the framework, WHO provides the vaccine to consenting at-risk individuals, health-care workers as well as ring contacts outside of clinical studies.[55,56] In a new flare-up of EVD in Guinea in March 2016, after the end of the West African epidemic was declared, the framework was utilized to vaccinate over 1500 individuals including children from the age of 6, and no EVD cases were reported in the vaccinated population.[57] Another 3481 people were vaccinated during the 9th EBOV outbreak in the DRC in May and June 2018.[58] During the current 10th EBOV outbreak in the DRC, which was declared on August 1st, 2018 and has claimed the lives of 2592 humans as of July 21st, 2019, VSV-EBOV has been administered a total of 171,052 times since the initiation of vaccination seven days later.[59]

In addition to WHO, MSF is also providing the vaccine using the ring vaccination strategy and has assessed vaccine efficacy in a clinical trial including 500 individuals from the age of one. The trial was reportedly completed on November 30, 2018 (clinicaltrials.gov, NCT03161366) and the results are awaited with great anticipation. Meanwhile, WHO recently reported highly encouraging preliminary results of the ring vaccination regimen applied during the recent DRC EBOV outbreaks including a total of 93,965 vaccinated individuals and reported an estimated vaccine efficacy of 97.5%, thus strongly supporting the findings of the phase III trial.[60]

Stability of the vaccine

In the context of the deployment of VSV-EBOV in the field setting, the storage of the vaccine, which must be kept at a temperature of −70°C maximum, remains a challenge. The establishment and maintenance of cold chains in often rural surroundings with suboptimal infrastructure has been difficult.[61] To date, stability data remain scarce, yet one study found that the vaccine stayed stable with comparable viral titers for 7 days when stored at +4°C.[62] In a guinea-pig challenge model, vaccination with VSV-EBOV conferred protection from disease even when stored in various suboptimal conditions over 7 days (4°C, room temperature, 32°C, or three cycles of freeze-thawing from −80°C to room temperature), and only the vaccine kept at 32°C showed a significant reduction in virus titer.[63] The data indicate that short-term

interruptions of the cold chain may be tolerated without affecting vaccine efficacy.

Correlates of protection and duration of immune responses

Understanding the correlates of protection in EVD remains a challenge. There is still no biomarker that associates with EBOV vaccine efficacy. The identification of EBOV-associated correlates of protection is challenging as markers can only be defined when EBOV incidence is sufficiently high such as during outbreaks. While the "Ebola Ça Suffit!" trial provided strong evidence for high protection of VSV-EBOV, blood samples to analyze immunogenicity and protection markers could not be generated during this trial.

First insights into possible correlates of protection have been provided by assessing immune responses of survivor and fatal cases from EBOV-infected individuals following the West African EVD epidemic. One study demonstrated that fatal cases were associated with an increased expression of the exhaustion markers CTLA-4 and PD-1 on $CD4^+$ and $CD8^+$ T cells.[64] A transcriptomic analysis of blood RNA from EBOV-infected patients showed elevated expression levels of genes associated with interferons and acute phase signaling pathways.[65] Lower levels of inflammation correlated with virus clearance and robust EBOV-specific T-cell responses. Although these novel data provide insight into innate and adaptive immune responses, their contribution to protection in humans is still not defined. Notably, correlates of protection may differ between natural EVD infection and vaccine-induced immune responses.

Studies comparing immune responses in EVD survivors are currently ongoing (César Muñoz-Fontela, Florian Klein personal communication).

Animal models can be crucial to decipher immune response mechanisms important for protection. In the past, guinea pig as well as non-human-primate EBOV models were used to unravel protective mechanisms. Andrea Marzi and colleagues comprehensively analyzed EBOV challenges in cynomolgus macaques after vaccination. Depletion methods, in which CD4+ and CD8+ T cells as well as B-cells were blocked, revealed a critical role for EBOV GP-specific antibodies.[66] The pivotal role of VSV-EBOV-induced innate immunity in conferring early antiviral control and generating effective humoral immune responses was demonstrated in an NHP challenge model. Transcriptional whole blood profiling from vaccinated animals challenged at different timepoints (28, 21, 14, 7 and 3 days p.v.) uncovered that genes associated with antiviral innate responses were upregulated at early timepoints, and were delayed in an animal that did not survive challenge at day 3 p.v.[50,67]

The collection of samples obtained during the West African epidemic and the ongoing outbreak in the DRC together with numerous individuals vaccinated with VSV-EBOV can now generate a highly valuable body of information to fill critical knowledge gaps regarding mechanisms crucial for immune protection. A first meta-analysis has now been published by Gross and colleagues summarizing antibody responses of all clinical trials in which VSV-EBOV was tested.[68] All studies observed induction of EBOV GP-specific antibody responses following immunization with VSV. Antibody responses developed at 14 days p.v., peaked around day 28 and remained detectable for at least 2 years.[28,29,40] Besides evaluating humoral responses, clinical trials also evaluated all other arms of immune responses. One study focused on T follicular helper (TFH) cells and observed a correlation with antibody titers.[69] Cellular immune responses were observed in the cohort receiving a dose of 2×10^7 PFU. Here, EBOV GP specific T-cells secreted mainly CD107a and TNFα, while lower dose groups showed antigen-specific T-cell responses.[70] Recent advances in our understanding of the innate immune system and the use of systems biology approaches are beginning to reveal the fundamental mechanisms by which the innate immune system orchestrates protective immune responses to vaccination. We investigated innate immune responses following VSV-EBOV immunization and our data revealed an innate immune signature that correlated with antibody responses in the Hamburg VSV-EBOV vaccine trial.[71] In addition, changes in expression levels of circulating miRNAs have been associated with increased EBOV GP-specific antibody titers. Furthermore, innate immune signatures were assessed in vaccinees from Europe and Africa, revealing an early induction at day 1 p.v. of chemokines and cytokines, associated with monocyte functionality.[72]

Comprehensive evaluations of immunogenicity following VSV-EBOV vaccination are still ongoing. Systems vaccinology approaches in which clinical, immunological, transcriptomic and metabolomic data from clinical vaccine studies can now be integrated and analyzed with data from survivors will be instrumental to further identify EBOV vaccine correlates of protection.

Experience in use as post-exposure prophylaxis

VSV-EBOV is intended for use as a prophylactic vaccine; however, its implementation as a post-exposure prophylaxis is intriguing, as a swift identification and vaccination of EVD-affected individuals – if effective – would contribute to a timely containment of future outbreaks. In NHPs, VSV-EBOV was fully protective when given 7 days before challenge and ameliorated disease when given 3 days pre-exposure.[50] In mice, vaccination with VSV-EBOV 24 h post-infection (p.i.) conferred complete protection, while guinea pigs only showed partial protection from challenge when vaccinated 24 h pre- to 24 h post-exposure. Lastly, in NHP, 50% protection was conferred in animals vaccinated 20–30-min post-exposure.[73] In line with this finding, a separate study described an overall 50% protection from EVD when NHP were vaccinated either 1 h or 24 h p.i. or when vaccinated twice, at 1 h and 24 h p.i. with half the vaccine dose, respectively. Surprisingly, however, post-exposure prophylaxis with an rVSV-vectored vaccine against Marburg virus conferred similar protection against EVD, and the authors argued that VSV-driven innate immune activation as well as filovirus-specific immune responses may play a role in protection from EVD.[74]

Of note, compared to the disease course in humans, the onset of clinical disease is faster and its manifestation more severe in the NHP model, with a lethality of close to 100%. When NHPs were challenged with Sudan ebolavirus (SUDV), which leads to lethal disease in NHP later than does EBOV,

100% of NHP were protected when vaccinated with rVSV-SUDV 20–30 min p.i.[75] The efficacy of post-exposure prophylaxis may thus well be higher in humans than in the NHP model.[76]

In humans, VSV-EBOV was first used as an emergency post-exposure vaccine in 2011, long before entering clinical trials, and has been used in a total of seven individuals with occupational exposure to EBOV. Here, the vaccine was administered 24 h to 3 days post-exposure and none of the individuals developed EVD.[77–79] It is of course unknown whether, and to what extent, post-exposure prophylaxis prevented the development of EVD, but albeit that these case reports are anecdotal in nature, they are encouraging and provide the basis for current EVD post-exposure recommendations.[76]

Vector-specific immunity

Although pre-existing anti-VSV immunity is rarely found in humans,[8] anti-vector immunity after vaccination with a VSV vector vaccine might impair vaccine efficacy of future VSV-based vaccines and prime-boost regimens. While data investigating pre-existing immunity remain scarce, we recently demonstrated that vaccination with VSV-EBOV induced both vector-specific humoral and cellular immune responses.[80] The VSV-specific antibody responses generated in humans in this study following vaccination with VSV-EBOV were non-neutralizing and correlated with EBOV GP-specific antibody responses.[80] With regard to the potential simultaneous use of VSV-EBOV and VSV-based Lassa virus (LASV) vaccines in West Africa, Marzi et al. vaccinated cynomolgus macaques with VSV-EBOV 90 days after having received a protective dose of the Lassa vaccine VSVΔG/LASVGPC. Despite having significant anti-VSV antibody titers before vaccination with VSV-EBOV, the vaccination conferred full protection from EBOV challenge, suggesting that a repeated vaccination with VSV-vectored vaccines may be efficacious.[81]

Future perspectives for VSV-based Ebola vaccines

While clinical trials of VSV-EBOV are still ongoing, efforts to create more attenuated second-generation rVSV-EBOV vaccines are already underway with the goal to reduce reactogenicity while maintaining immunogenicity. In one approach, VSV GP was substituted with an EBOV GP exhibiting the mutation F88A which impairs cell entry. VSV*ΔG(EBOV-GP_{F88A}) induced immunogenicity in guinea pigs comparable to vaccination with rVSV expressing propagation-competent EBOV GP. However, the vaccine construct was found to be genetically unstable and quickly reverted back to a more infectious variant when passaged. Further mutations may, therefore, have to be introduced in order to maintain propagation restriction.[82] N4CT1-EBOVGP1 is another attenuated rVSV-EBOV vaccine candidate. The rVSV vector is attenuated by combining a translocation of the N protein from position 1 to 4 (N4) with a truncation of the VSV-G cytoplasmatic tail (CT), and expresses the EBOV GP from the EBOV-Mayinga strain from the first position of the rVSV genome.[83] The vector has previously been tested as an HIV vaccine candidate in phase I clinical trials and was well tolerated.[19,84] N4CT1-EBOVGP1 vaccinated mice developed both humoral and cellular immune responses and guinea pigs and macaques were completely protected from EBOV challenge, mounting antibody responses in the latter by day 14 p.v.[83] Importantly, vaccinated primates were also protected against heterologous challenge with EBOV-Makona.[85] N4CT1-EBOVGP1 is licensed by Profectus BioSciences, a biotech company that has specialized in vaccine development and uses the attenuated rVSV vector in a number of vaccine candidates. N4CT1-EBOVGP1 is now being evaluated in a phase I dose-escalation trial (NCT02718469).

Marburg virus

Marburg hemorrhagic fever, caused by Marburg virus (MARV) of the genus *Marburgvirus*, is characterized by its high case-fatality rate. Also belonging to the family of *Filoviridae*, MARV shares a lot of characteristics with EBOV. However, historically less frequent outbreaks with fewer affected individuals have occurred.[86] It both poses a great potential public health threat as an emerging virus and is listed as a category A bioterrorism agent by the Center for Disease Control (CDC), making vaccine development a priority. Despite decades of research, no licensed vaccines or therapies are available to date. However, ample preclinical data on rVSV-vectored MARV vaccine candidates have been generated – often alongside the EBOV vaccine candidates, both as a pre-exposure prophylactic as well as a post-exposure vaccine (reviewed in[15,87,88]).

Analogous to its EBOV counterpart, rVSV-MARV vaccine candidates utilize the immunogenic MARV GP and have shown tolerability as well as efficacy in various animal models, including the NHP model. As a preventive vaccine, VSVΔG/MARVGP, which uses GP strain Musoke as the expressed antigen, was shown to completely protect cynomolgus macaques 28 days p.v. from lethal MARV challenge, and even when challenged almost 4 months after initial vaccination and with a different MARV strain (Popp).[24]

A subsequent study further demonstrated that the vaccine was capable to confer cross-protection when NHP were challenged with the more virulent strain Angola, as well as the genetically distant Ravn strain with an amino acid deviation of around 20%. In this study, a dose of 2×10^7 PFU was administered; high anti-MARV IgG titers developed and rVSV viremia was detected around 3 days p.v., closely mirroring the observations made for VSV-EBOV. The primates did not develop any signs of clinical illness or abnormalities in hematology or blood chemistry values.[89]

Peri- and post-exposure prophylaxis efficacy have been evaluated in animal models. A vaccination 20–30 min p.i. fully protected rhesus macaques from homologous challenge with >10,000 LD_{50} doses of MARV, and administration 24 h and 48 h post-exposure protected 5/6 and 2/6 macaques, respectively, suggesting efficacy – albeit limited – as a post-exposure treatment, even at high exposure doses.[90,91] In contrast, when two vaccine candidates expressing MARV-Angola GP were tested for post-exposure efficacy 20–30 min after homologous MARV challenge, survival rates were only 25–75% depending on the challenge dosage and attenuation of the vector used, probably due to the higher pathogenicity as well as shorter incubation period of the MARV-Angola variant, which is of high clinical importance, as the MARV-Angola strain has been responsible for the worst outbreak of Marburg hemorrhagic fever so far.[92] Notably, all surviving

animals had mounted anti-MARV specific IgG antibody responses by day 10, indicating that post-exposure treatment may confer protection by delaying disease progression long enough for the infected subject to develop sufficient immunity.[91,92]

The durability of post-vaccination immunity was furthermore assessed by monthly measurements of anti-MARV-GP antibodies in initially VSVΔG/MARVGP vaccinated macaques. All animals developed strong humoral responses that remained overall stable over the course of 13 months. When the macaques were then challenged with homologous MARV-Musoke, no animal developed clinical signs of illness, demonstrating efficacy of the vaccine for at least a year.[93] Taken together with data from other studies, the strong protective capacity of rVSV-MARV seems to be primarily mediated by a strong humoral immune response.[24,89,92,94]

Combination of rVSV-vectored filovirus vaccines

While the largest EVD outbreaks to date have been caused by EBOV, EVD outbreaks caused by other ebolavirus species have also been described, and MARV and EBOV outbreaks occur in overlapping geographic regions. Cross-protection of VSV-EBOV and -MARV against heterologous strains has been demonstrated as outlined above;[50,89] however, limited or no cross-protection might exist between the more distantly related species and genera.[24,95,96] Therefore, a combination of rVSV-vectored filovirus vaccines would be desirable and has been evaluated.

After being vaccinated with a multi-component vaccine consisting of rVSV-ΔG-EBOV-, -MARV- and -SUDV-GP, macaques survived challenge with either of the respective species or TAFV. Monkeys challenged with MARV, EBOV or TAFV did not show clinical signs of disease, while those challenged with SUDV did. The authors argued that this observation could be due to slower replication kinetics of rVSVΔG-SUDV or the differential affinity of GP to antigen-presenting cells. They, therefore, tested a two-stage vaccination administering rVSVΔG-SUDV two weeks prior to vaccination with rVSVΔG-EBOV and -MARV, which resulted in complete protection from challenge with no clinical evidence of infection both when challenged with SUDV and subsequently back challenged with MARV.[97] In another study, the rVSV-N4CT1 vector expressing either EBOV, SUDV or MARV GP from the first position of the rVSV genome was administered to macaques as a trivalent vaccine and none of the animals succumbed to the disease after challenge with any of the pathogens. However, a subset of vaccinated macaques did develop mild signs of disease as well as low levels of filovirus viremia, especially after challenge with EBOV, when this attenuated vector was used.[98] The same vector is currently employed in efforts to develop a multivalent vaccine candidate against MARV, SUDV, EBOV and additionally Lassa virus.[99]

Lassa virus

The arenavirus LASV is one of the primary causal agents of hemorrhagic fevers worldwide with more than 300,000 estimated infections recorded annually in West Africa, where it is endemic.[100] It is a rodent-transmitted disease and, while disease courses are highly diverse depending on LASV clade, morbidity is significant.[100] Since January 2019, Nigeria has been facing an ongoing outbreak of Lassa fever with 526 confirmed cases of LASV infections and 121 deaths (case-fatality rate 23%, Nigeria Center for Disease Control (NCDC) as of March 31, 2019).[101] Due to its widespread geographic distribution, high incidence and the lack of a preventive vaccine or approved therapies, LASV has been identified as an emerging virus for which R&D efforts are given very high priority by multiple international professional organizations, including the Coalition for Epidemic Preparedness Innovations (CEPI) and WHO.[102]

Analogous to rVSV vectors expressing MARV and EBOV GP, Drs. Feldmann and Stroeher had described a LASV Glycoprotein C (GPC) expressing rVSV, VSVΔG/LASVGPC (clade IV, isolate Josiah), with no detectable pathogenicity in mice.[17] As the NHP model is the most relevant animal model for LASV infection, efficacy of a vaccine dose of 2×10^7 PFU was assessed in macaques (n = 4).[103] VSVΔG/LASVGPC was well tolerated and no viral shedding occurred after vaccination. Hematology and blood chemistry analysis found a slight thrombocyte decrease as well as a slight elevation of ALT in 3/4 animals. 100% of the animals remained asymptomatic after lethal LASV challenge 28 days after vaccination in contrast to controls, which succumbed to infection, and high LASV-specific antibody titers as well as moderate T-cell responses could be detected. Immunity was not sterile and animals developed viremia that was cleared by day 10.[103] Viremia was, however, not observed in consecutive experiments.[81,104] Another vaccine construct with VSV expressing LASV nucleoprotein (NP) meanwhile only showed limited protection and was not pursued further.[104]

The genetic heterogeneity observed between LASV clades raises concerns on whether VSVΔG/LASVGPC can act as a universal vaccine and this question was examined in subsequent studies. In inbred guinea pigs as well as NHPs, vaccination with VSVΔG/LASVGPC resulted in full protection against homologous challenge as well as against challenge with heterologous LASV clade IV isolates 28 days p.v. Again, challenge of inbred guinea pigs with the clade I isolate Pinneo, one of the most genetically divergent isolates compared to Josiah, did not result in clinical disease, while it has to be noted that animals vaccinated with an irrelevant control vector did develop symptomatic disease, but were able to clear infection.[104] Further work to elucidate Lassa cross-clade immunity is currently ongoing.[105]

A better understanding of the durability of VSVΔG/LASVGPC is needed to estimate its potential benefit as a vaccine candidate. Stein and colleagues, therefore, sought to examine how early and how long VSVΔG/LASVGPC can confer protection. In a guinea pig-adapted (GPA) LASV model, animals vaccinated with 1×10^6 PFU VSVΔG/LASVGPC were challenged at a timepoint between 7 and 355 days p.v. As early as 7 days p.v. the vaccine provided full protection from clinical disease; however, immunity was not sterile. Antibody responses peaked on day 51 and were sustained until the final sampling date one year later. When challenged 355 p.v., the guinea pigs did develop signs of disease, but 71% of animals were still protected from death.[106]

In recent developments, CEPI furthermore awarded considerable research funds to develop, manufacture and ultimately stockpile a Lassa virus vaccine based on the above mentioned rVSV-N4CT1 platform.[107]

While further research including additional studies in NHPs is warranted, the data suggest that VSVΔG/LASVGPC may be a universal vaccine candidate that can confer early and lasting immunity.

Crimean-Congo hemorrhagic fever virus (CCHFV)

Based on the promising results obtained from the studies with rVSV vaccine candidates expressing filo- and arenavirus GP, a replication-competent rVSV expressing glycoprotein precursor (GPC) of CCHFV, likewise a hemorrhagic fever virus, has recently been developed and tested in an immunocompromised (STAT-1 knock-out) mouse model. In this study, a prime-only and a prime-boost regimen (days 0 and 7) were compared to mock vaccination, and immunogenicity, as well as protection from challenge with CCHFV 35 days after prime vaccination were assessed. Both groups receiving either one or two injections developed CCHFV-GP binding as well as virus neutralizing antibodies. The prime-boost group showed milder signs of infection and 100% of animals in both prime-only and prime-boost groups survived the challenge, while none of the mock-vaccinated did.[108]

Nipah virus

The Henipavirus Nipah virus (NiV) is a zoonotic pathogen that can cause severe pulmonary and neurologic disease with a high case-fatality rate and has been responsible for almost yearly outbreaks on the Indian subcontinent. The NiV vaccine portfolio currently under development includes rVSV-vectored Nipah vaccines, which utilize rVSV expressing two different structural proteins as antigens, the NiV fusion (F) and attachment (G) glycoproteins – two surface proteins that together are required for viral cell entry. The rVSV vector expressing either antigen has been described in two different models, one with the insertion of either protein after VSV-G (VSV-G/F(NiV)), the other in place of the VSV glycoprotein (rVSVΔG-G/F(NiV)). In mice, intranasal (i.n.) administration of rVSV-G(NiV) and/or -F(NiV) induced neutralizing Ab (nAb) formation, and highest nAb formation was observed when rVSV-G(NiV) alone or in combination with rVSV-F(NiV) was given. The rVSVΔG vector was evaluated using i.m. vaccination and likewise induced nAb, especially when rVSVΔG-G- and -F(NiV) were used in combination.[109] Of note, the combined administration of rVSVΔG-G/F(NiV) was found to be lethal when given intranasally in 5/8 postnatal mice in a later study, while the individual administration of either vaccine candidate did not cause any symptoms, even when directly injected into the brain.[110]

Vaccination with rVSVΔG-G- or -F(NiV) was demonstrated to be fully protective against NiV challenge in a Syrian hamster model, where none of the animals developed disease.[111] Similarly, the vector VSV-EBOV, complemented with NiV F or G protein downstream of EBOV-GP, was assessed in the hamster model and conferred the same level of protection from disease.[112] Importantly, while the vaccine was administered 28 days prior to challenge in this study, a follow-up study additionally demonstrated peri-exposure protection. Full protection was achieved until one day before vaccination and decreased to 17% until one day after challenge, while administration three days after challenge did not show protection.[113] Interestingly, similarly to observations made by Marzi et al. in an EBOV post-exposure vaccine model,[74] an rVSV vector vaccine control also mediated partial protection when given one day pre-challenge.[113] Antibody generation was associated with protection[113] and a serum-transfer experiment confirmed that protection was antibody-dependent.[112] A simultaneously assessed rVSV-ΔG-EBOV vector expressing NiV nucleocapsid protein (N) was shown to be inferior to the F and G variants.[112] The more immunogenic rVSV-ΔG-EBOV-G(NiV) was subsequently demonstrated to be completely protective in African Green Monkeys, described as the most appropriate animal model for NiV infection.[114,115]

MERS- and SARS-coronaviruses

SARS as well as MERS constitute severe pulmonary infections that are caused by SARS- and MERS- coronaviruses (CoV), respectively. A severe outbreak of SARS-CoV, which originated in China in 2002, spread swiftly and ultimately affected over 8000 humans within a few months.[116] While the virus has been contained, its potential to reemerge and cause new outbreaks remains high, and the rVSV platform has been employed to create SARS-CoV vaccine candidates for such a future scenario. VSV-S expresses the immunogenic spike protein (S) of SARS-CoV between the G and the L gene of VSV. In a mouse model, vaccination with VSV-S induced higher levels of neutralizing antibodies than immunization with SARS-CoV and conferred protection as measured by low or undetectable SARS-CoV viral loads in the respiratory tract, even when challenged 4 months p. v. Using antisera from VSV-S vaccinated mice in naïve mice, it could further be shown that the protective effect was mediated by antibodies.[117] This vaccine candidate was then attenuated by replacing VSV G with SARS-CoV S, resulting in VSVΔG-S. Immunogenicity of the replication-defective vector was surprisingly greater than the immunogenicity of VSV-S when applied i.m., an effect the authors argued that could be due to a higher expression of SARS-CoV S protein in VSVΔG-S, among others. In an analogous manner, VSVΔG was later used to create a MERS-CoV vaccine by expressing the MERS-CoV spike protein (S), and VSVΔG-MERS displayed immunogenicity in rhesus monkeys.[118] To date, no human clinical trials have been reported for VSV-based CoV vaccines and the WHO roadmap for MERS-CoV research and product development does not currently include rVSV-vectored vaccines in the port- folio of vectored vaccines.[119]

Zika virus

rVSV vectors have also been employed in designing vaccine candidates against Zika virus (ZIKV), a flavivirus closely related to Dengue, West-Nile, Japanese encephalitis and Yellow Fever viruses, which has been associated with severe neurological

disorders such as microcephaly in newborns and Guillan-Barré Syndrome. In 2015–2017, the spread of ZIKV infections received attention when it rapidly disseminated from Brazil throughout the Americas, where no autochthonous case of ZIKV infection had been described before 2015.[120,121]

rVSV vaccine vector expressing diverse ZIKV antigens have been described in proof-of-concept studies. In 2017, an rVSV vectors that entails a mutated M protein as an attenuation factor (VSVm) and expresses either Zika envelope (ZENV) alone or ZENV containing the precursor to membrane (prM) proteins (ZprME) was described. The constructs remained replication competent. In mice, vaccination did not trigger any overt pathology and VSVm-ZprME vaccination did induce neutralizing antibody titers.[122] As ZIKV infection does not lead to disease in adult mice, challenge studies were performed in offspring of vaccinated mothers and, therefore, statements about the efficacy of the vaccine were limited.

In another approach, the attenuated VSV-EBOV was used as a vector to express ZprME or prM and soluble envelope proteins as antigens (ZprMsolE) and efficacy was tested in type I interferon receptor deficient mice (IFNAR$^{-/-}$). The ZIKV vaccine candidates did not result in pathogenicity in mice, however, unexpectedly vaccination with VSV-EBOV control vector led to severe clinical symptoms in the immunocompromised mice, thus the VSV-EBOV-based ZIKV vaccine constructs appear to be more attenuated than the original VSV-EBOV in this study. Mice challenged with ZIKV 28 days p.v. were fully protected from disease. Antigenicity of ZprME seemed to be superior to ZprMsolE and 50% and 100% protection was achieved when mice were vaccinated with rVSV-ZprME 1 and 3 days pre-challenge, respectively. Interestingly, vaccination with the vaccines was sufficient to also protect outbred CD1 mice from EBOV challenge.[123]

In a study assessing multiple ZIKV antigens in a VSV vector, which was attenuated via a point mutation in the VSV L protein, combining the ZIKV prM and E proteins with the nonstructural NS1 protein showed robust humoral and cellular responses in immunocompetent mice, as well as protection from disease in IFNAR$^{-/-}$ mice.[124]

Furthermore, a VSV vector expressing the ZIKV capsid protein was also shown to be immunogenic with dominating cellular immune responses and reduced viral replication in the spinal cord and brain tissues of immunocompetent mice.[125] No human clinical trials using VSV-based ZIKV vaccine candidates have been initiated to date.

Conclusion

The rVSV vaccine platform exhibits many advantages as a vaccine vector backbone for the expression of viral antigens and has been tested as a vaccine candidate against a multitude of emerging infections. As a result of the recent clinical phase I-III trials of VSV-EBOV, we have now gained a significant amount of data in humans, which encouragingly suggest a favorable safety profile of the vaccine candidate as well as swift protection after a single immunization. VSV can readily be attenuated while remaining immunogenic. While the comprehensive details on VSV vaccine-mediated protective immunity have yet to be elucidated, the data generated to date support the premise that protection appears to be primarily inferred by antibody responses. However, cellular immunity is simultaneously induced. In addition, the fact that recombinants can be manufactured quickly with diverse antigens further adds to its value as a vaccine platform for outbreak pathogens. While we have focused here on WHO blueprint priority pathogens, a variety of VSV-based vaccine candidates have been described for other emerging or reemerging pathogens, including enterovirus 71,[126] Chikungunya,[127,128] West-Nile,[129] Dengue,[130] Severe fever with thrombocytopenia syndrome,[131] and Andes viruses.[132–134] Animal studies of VSV-based vector vaccines against MARV, LASV, NiV, ZIKV, and MERS- and SARS-CoV described above have yielded promising results and the progression of rVSV vaccine candidates to clinical trials may add to the portfolio of effective outbreak vaccines in the foreseeable future.

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