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Live virus vaccines based on a vesicular stomatitis virus (VSV) backbone: Standardized template with key considerations for a risk/benefit assessment ☆

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ABSTRACT

The Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG) was formed to evaluate the safety of live, recombinant viral vacci2;nes incorporating genes from heterologous viral and other microbial pathogens in their genome (so-called "chimeric virus vaccines"). Many such viral vector vaccines are now at various stages of clinical evaluation. Here, we introduce an attenuated form of recombinant vesicular stomatitis virus (rVSV) as a potential chimeric virus vaccine for HIV-1, with implications for use as a vaccine vector for other pathogens. The rVSV/HIV-1 vaccine vector was attenuated by combining two major genome modifications. These modifications acted synergistically to greatly enhance vector attenuation and the resulting rVSV vector demonstrated safety in sensitive mouse and non-human primate neurovirulence models. This vector expressing HIV-1 gag protein has completed evaluation in two Phase I clinical trials. In one trial the rVSV/HIV-1 vector was administered in a homologous two-dose regimen, and in a second trial with pDNA in a heterologous prime boost regimen. No serious adverse events were reported nor was vector detected in blood, urine or saliva post vaccination in either trial. Gag specific immune responses were induced in both trials with highest frequency T cell responses detected in the prime boost regimen. The rVSV/HIV-1 vector also demonstrated safety in an ongoing Phase I trial in HIV-1 positive participants. Additionally, clinical trial material has been produced with the rVSV vector expressing HIV-1 env, and Phase I clinical evaluation will initiate in the beginning of 2016. In this paper, we use a standardized template describing key characteristics of the novel rVSV vaccine vectors, in comparison to wild type VSV. The template facilitates scientific discourse among key stakeholders by increasing transparency and comparability of information. The Brighton Collaboration V3SWG template may also be useful as a guide to the evaluation of other recombinant viral vector vaccines.

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1. Introduction

Recombinant viral vectors provide an effective means for heterologous antigen expression *in vivo* and thus represent promising platforms for developing novel vaccines against human pathogens such as Ebola virus, human immunodeficiency virus (HIV), tuberculosis, and malaria [1–10]. Preclinical evaluation of

☆ The findings, opinions, conclusions, and assertions contained in this consensus document are those of the individual members of the Working Group. They do not necessarily represent the official positions of any participant's organization (e.g., government, university, or corporations) and should not be construed to represent any Agency determination or policy.

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such viral vector vaccines has indicated their potential for immunization and an increasing number of candidate vaccines are entering human clinical trials. Improving our ability to anticipate potential safety issues and meaningfully assess or interpret safety data from trials of such new viral vector vaccines will increase their likelihood of public acceptance should they be licensed [11–14].

The Brighton Collaboration (www.brightoncollaboration.org) was formed in 2000 as an international voluntary collaboration to enhance the science of vaccine safety research [15]. In recognition of these needs in this domain, the Brighton Collaboration created the Viral Vector Vaccines Safety Working Group (V3SWG) in October 2008. Analogous to the value embodied in standardized case definitions for Adverse Events Following Immunization (AEFI), the V3SWG believes a standardized template describing the key characteristics of a novel vaccine vector, when completed and maintained with the latest research, will facilitate the scientific discourse among key stakeholders by increasing the transparency and comparability of information. The International AIDS Vaccine Initiative (IAVI) had already developed an internal tool to assess the risk/benefit of different viral vectors under its sponsorship. The IAVI graciously shared this tool with the V3SWG for adaptation and broader use as a standardized template for collection of key information for risk/benefit assessment on any viral vector vaccines. This tool was aimed at identifying potential major hurdles or gaps that would need to be addressed during the development of vectored vaccines. The template collects information on the characteristics of the wild type virus from which the vector was derived as well as known effects of the proposed vaccine vector in animals and humans, manufacturing features, toxicology and potency, nonclinical studies, and human use, with an overall adverse effect and risk assessment.

The V3SWG anticipates that eventually all developers/researchers of viral vector vaccines (especially those in clinical development) will complete this template and submit it to the V3SWG and Brighton Collaboration for peer review and eventual publication in Vaccine. Following this, to promote transparency, the template will be posted and maintained on the Brighton Collaboration website for use/reference by various stakeholders. Furthermore, recognizing the rapid pace of new scientific developments in this domain (relative to AEFI case definitions), we hope to maintain these completed templates "wiki-" style with the help of Brighton Collaboration and each vectored vaccine community of experts [16].

1.1. Need for risk/benefit assessment of live virus vaccines based upon a vesicular stomatitis virus (VSV) backbone

Vesicular stomatitis virus (VSV), a negative sense RNA virus of the *Rhabdoviridae* family, has become a prominent tool as a vaccine vector against microbial pathogens [17]. Desirable properties of recombinant VSV (rVSV) include robust growth in approved, continuous mammalian cell lines and the inherent ability to elicit strong cellular and humoral immune responses. Importantly, some highly attenuated forms of rVSV show no signs of virulence in animals, and attenuated, replication competent forms of rVSV have now demonstrated safety and immunogenicity in multiple clinical trials, specifically HIV Vaccine Trial Network (HVTN) 087 and 090 [18]. In animals, pathogenicity and immunogenicity has been largely attributed to the VSV glycoprotein (VSV G) [19,20] with decreased or no infection achieved when the VSV G gene has been modified [21]. These factors, in combination with a very low seroprevalence of VSV in humans, support the use of rVSV as potential vaccine vectors, as discussed below.

1.1.1. Low seroprevalence in humans

While the natural hosts of VSV are insects and livestock, a few incident cases have occurred in humans as a result of high-risk occupational exposure (i.e. laboratory workers, farmers, veterinarians) [22,23]. Infected humans may be asymptomatic or may experience a mild febrile illness with symptoms lasting 2–5 days [23]. The low incidence of infection and disease results in an overall very low level of pre-existing immunity to the virus among the general human population. Areas of exception include rural communities of Central America where both predominant serotypes VSV-New Jersey (VSV-NJ) and VSV-Indiana (VSV-IN) are endemic [24,25]. Other areas of note include the enzootic regions of coastal Georgia where seroprevalence of humans to VSV-NJ was approximated at 30% in the early 20th century [26].

1.1.2. Gene expression

Viral vector vaccines should demonstrate stability of foreign gene expression to ensure high-level expression of the target antigen(s). VSV has a simple genome of 11 KB encoding five major proteins. Transcriptional attenuation of approximately 30% occurs at each successive gene junction resulting in a pronounced 3' to 5' gradient of gene expression [27–30]. Therefore, the genomic site of foreign gene insertion strongly influences antigen expression levels. Minimal conserved nucleotide sequences (transcription start and stop signals) are required for normal gene expression [31] and foreign gene inserts must be flanked by these sequence elements.

Although there are no apparent structural limitations on the size of foreign gene insert for the VSV vector, larger inserts appear to reduce the rate of viral replication in animal models. For example, rVSVGagEnv encoding both the HIV envelope (Env) and group specific antigen protein (Gag) contributing approximately 4.4 kilobases (kb) of additional genomic sequence, modestly reduced viral titers by threefold [32]. Since then, a larger insert of approximately 6 kb encoding Hepatitis C virus non-structural proteins (NS) has been expressed by a rVSV NJ vector, leading to a fivefold reduction in viral titer [33]. It is, however, also likely that some foreign gene products may further inhibit rVSV replication by other mechanisms such as biological activity, targeting and transport, or unforeseen toxicity.

1.1.3. Attenuation strategies

The pathogenicity of VSV has been attributed in part to the glycoprotein (VSV G), as virulence is dependent on the ability of G protein to bind cellular receptors, and mediate entry and fusion with endocytic vesicles to initiate the replicative cycle [34]. Due to pivotal roles in receptor binding and membrane fusion, it has been a target for attenuation of rVSV vector vaccines. Replacement of the G gene with that of another foreign gene product acting as a viral receptor can generate rVSVΔG pseudotypes with altered cell tropism, which may also have attenuating effects. Foreign glycoproteins expressed by these pseudotypes are prime targets for cell-mediated and humoral immunity [35,36]. Thus far, rVSV and rVSVΔG vectors expressing influenza hemagglutinin (HA) and Ebola/Marburg glycoproteins have demonstrated full protection against virus challenge and are non-pathogenic in mouse and non-human primate (NHP) disease models [37–44]. The strategy of using rVSV pseudo-typed with Ebola virus GP as a vaccine to combat Ebola virus induced disease has recently completed clinical testing and will be discussed in a separate vector analysis template due to the unique properties of the vector conferred by the Ebola virus GP protein as sole virus receptor. *In vitro* and *in vivo* attenuation of rVSV has also been demonstrated by truncation of the cytoplasmic tail (CT) of the G protein from 29 amino acids found in nature, to only 9 or 1 amino acids (CT9 and CT1 respectively)

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[17,42,45]. It is generally thought that this attenuation mechanism acts by impairing the interaction of the G CT with underlying viral core proteins, thereby reducing the efficiency of virus particle maturation and budding.

Another major approach to rVSV attenuation relies on down-regulation of expression of one or more key viral structural proteins. This attenuation strategy has been demonstrated for rVSV by translocation of the N gene further away from the 3' transcription promoter to positions 2, 3 and 4 in the genome [28,29]. The resulting step-wise reduction in N protein expression leads to corresponding incremental reduction of viral replication *in vitro* and reduced pathogenesis in a natural host [30].

Attenuation by either CT truncation or N gene translocation separately could not provide sufficient reduction in neuropathology in stringent murine and NHP neurovirulence (NV) models to support testing of rVSV as a vaccine vector in humans [46–48]. However, when both forms of attenuation were combined there was a dramatic and synergistic increase in vector attenuation, almost completely eliminating clinical and microscopic pathology following intra-cranial injection of mice and NHPs [47,49,50].

One additional attenuation mechanism relies on either mutation or deletion of amino-acid 51 of the VSV M protein These VSV M mutants grow quite robustly in cell culture but demonstrate a marked reduction of virulence *in vivo*. It is thought that the attenuating mutation(s) reduce the ability of virus to shut down host innate immune responses which normally restrict virus growth *in vivo* [51–53].

1.1.4. Post exposure protection

Studies using rVSVΔG vectors expressing Ebola and Marburg virus glycoproteins achieved post-exposure prophylaxis in both rodent and NHP models [20]. If administered in one dose within 24 h of virus challenge, 50–100% of both guinea pigs and mice were protected. Similarly, there was 50% protection of NHPs if treatment was administered within 30 min of challenge.

1.1.5. Clinical trials

A live viral vaccine safety standard for all licensed vaccines requires assessment of viral NV by intracranial inoculation of NHPs with the vaccine [54,55]. Vaccines for measles, mumps, yellow fever, polio and others have all been assessed for NV by this method [56–59]. A pilot NV study in NHPs demonstrated that prototypic rVSV vectors expressing HIV gag and env were not adequately attenuated for clinical evaluation [48]. However, extensive testing in mouse NV studies and two additional, sequential NHP NV studies led to the identification of rVSV vectors that were safe for clinical testing [49,50]); one of these highly attenuated vectors known as rVSVN4CT1gag1 was selected for a first in man clinical trial. The rVSVN4CT1gag1vector was attenuated by translocation of the N gene to the 4th position in the genome (N4), truncation of the G protein CT to a single amino acid (CT1) and the gag gene was located in the 1st position of the genome (gag1) to maximize gag protein expression. The rVSVN4CT1gag1 vector has now demonstrated safety and immunogenicity in phase 1 clinical trials [18] and the rVSVN4CT1 expressing Ebola virus GP is on a clinical development pathway as a candidate Ebola virus vaccine [41].

To provide clinical trial materials (CTM) for Phase 1 studies, an HIV-1 vaccine production process was developed in a 10L bioreactor under good manufacturing practices (GMP). An approved Vero cell line was used as substrate for vaccine vector amplification. Following infection, culture medium from infected cells was harvested once cell cytopathology was extensive (80–100%), and centrifuged to remove cellular debris. This unprocessed harvest material (UHM) was then conditioned with a virus stabilizer at a final concentration of 7.5% sucrose, 3.8 mM KH_2PO_4, 7.2 mM K_2HPO_4 and 5 mM L-Glutamate (SPG) and passed through an anion exchange membrane which binds rVSV particles. The membrane was then rinsed to remove cellular proteins, and DNA and virus particles were eluted in a high salt buffer. The high salt eluate was exchanged with a low salt phosphate buffer suitable for injection by a process of tangential flow ultra-filtration. The resulting virus preparation was then formulated with SPG and 0.2% hydrolyzed gelatin as additional virus stabilizer, sterile-filtered, and dispensed in vials as drug product (also known as CTM). CTM was stored frozen at −70 °C to −80 °C until ready for injection. CTM material generated by this process (or equivalent material generated by the same process) underwent toxicology testing in rabbits under GMP. Data from the toxicology study, the results of compendial safety tests performed at all key stages of vaccine manufacturing, and all data from pre-clinical development and safety testing of the rVSVN4CT1gag1 vector, were submitted to the FDA as part of an investigational new drug (IND) application in 2011. The FDA approved the rVSVN4CT1gag1 vector for clinical evaluation, and enrollment for HVTN 090, a Phase 1, double blinded, placebo controlled clinical trial began in October 2011, marking the first time an rVSV vaccine vector was administered to healthy trial participants. Data from this first in human trial have now been published [18]. The rVSVN4CT1gag1 vector has also demonstrated safety and immunogenicity in a second HIV-1 Phase 1 clinical trial as part of a pDNA prime, rVSV boost, vaccination regimen (HVTN 087: http://clinicaltrials.gov/).

1.1.6. Future directions

The safety and immunogenicity of the rVSVN4CT1gag1 vector in animal models and in clinical trials has demonstrated the potential of rVSV vectors targeting other infectious diseases. Robust and stable gene expression, a safe, attenuated phenotype, and induction of foreign antigen-specific immune responses, support further development of rVSV and other vesiculoviruses as platforms for vaccine development.

1.2. Methods for developing, completing, and reviewing the standardized template

Following the process described earlier, [60] as well as on the Brighton Collaboration Website (http://cms.brightoncollaboration.org:8080/public/what-we-do/setting-standards/case-definitions/process.html), the Brighton Collaboration V3SWG was formed in October 2008 and includes ~15 members with clinical, academic, public health, regulatory and industry backgrounds with appropriate expertise and interest. The composition of the working and reference group, as well as results of the web-based survey completed by the reference group with subsequent discussions in the working group, can be viewed at http://www.brightoncollaboration.org/internet/en/index/workinggroups.html. The workgroup meets via emails and monthly conference calls coordinated by a secretariat [15].

The V3SWG invited a VSV expert, David K. Clarke (DKC), who has been intimately associated with the development of vaccines based on highly attenuated rVSV vectors, to complete the template. The draft was then reviewed by the V3SWG. DKC updated the template with new information prior to publication. The resulting template is submitted as a guideline for evaluating the current issues in development of vaccines based on replicating VSV vectors.

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2. Standardized template (Table 1)

Risk/Benefit Assessment for Vaccine Vectors			
1. Basic Information	**Information**		
1.1. Author(s)	David K Clarke, PhD		
1.2. Date completed/updated	April 2, 2010/ March 20, 2013/January 8, 2015		
2. Vaccine Vector information	**Information**		
2.1. Name of Vaccine Vector	Recombinant vesicular stomatitis virus (rVSV)		
2.2. Class/subtype	Live-attenuated (attenuated replication competent viral vector)		
2.3. Proposed route of administration	Intra-muscular (IM)		
3. Characteristics of wild type agent	**Information**	**Comments/Concerns**	**Reference(s)**
3.1. Please list any disease(s) caused by wild type, the strength of evidence, severity, and duration of disease for the following categories:			
• In healthy people	Infection of humans with wild type VSV (wtVSV) can cause a mild flu like disease (usually without vesicle formation), resolving in 3–5 days without complications	The frequency of natural infection with wtVSV in humans is generally very low but in some endemic areas of Central and South America, 20–30% of the population are sero-positive. Some infections may be asymptomatic	
• In immunocompromised	Not known in humans	VSV is sensitive to IFN-α/β. Studies in mice indicate the IFN response is responsible for control of wtVSV. An intact **innate** immune response will likely exert control of VSV	[61]
• In neonates, infants, children	Unknown in neonates and infants. Disease potential in children seems to be the same as that for adults	Young children seroconvert in endemic regions of the world such as South and Central America	[62]
• During pregnancy and in the unborn	Unknown		
• Are there any other susceptible populations	Unknown		
• Animals	Wild type VSV causes disease in livestock. The disease is typified by vesicular lesions at bite sites around the mouth, nose, teats and coronary bands of the hooves. The disease in livestock is not considered severe, and the lesions usually resolve in 10–11 days without complication. Rodents and other small mammals are also susceptible to infection	The virus is most commonly transmitted by biting insects such as sand-flies, black-flies and mosquitoes. Some limited animal-to-animal transmission may occur through direct contact with vesicular lesions	
3.2. Is there any known evidence of neurological or cardiac involvement of the wild type agent?	There is no reproducible evidence of cardiac or neurological involvement following natural infection with wtVSV	The wtVSV can cause a severe neurological disease if directly injected into the brain of livestock, primates and rodents. wtVSV can also spread to the brain causing disease and in some cases death following intra-nasal (IN) and intra-venous inoculation of mice	**Johnson,** [63]

Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors			
3.3. What is known about the types of human cells infected and the receptors used in humans and animals?	wtVSV can infect a range of immortalized human cells *in vitro*. It is generally believed that the cellular receptor for VSV is ubiquitous. At one time phosphatidyl-serine was thought to be the major receptor for VSV; however, more recently the receptor has been identified as the cell LDL receptor. Early research speculated that tissue associated monocytes may be one of the susceptible cell types in humans	Monocytes have been identified as a major class of infected cells following IM injection of rodents. Sub-populations of macrophage in the lymph nodes have specific features that make them more permissive for VSV replication. Following intra-nasal (IN) inoculation of mice the virus can replicate in nasal epithelia, and spread to the brain infecting neurons, astrocytes, glial and ependymal cells. The virus can also spread to and infect cells in the lungs after IN inoculation	D. Cooper, Wyeth/Pfizer; unpublished observations [64–66]
3.4. Does the agent replicate in the nucleus?	No	VSV replicates in the cell cytoplasm	
3.5. What is the risk of integration into the human genome?	Very low probability event (if not impossible)	The VSV RNA genome replicates in the cytoplasm, and is always closely associated with the virus nucleocapsid protein. Integration of any viral gene product would have to occur through endogenous reverse transcription of a viral mRNA, followed by translocation into the nucleus and integration into the host genome	[67]
3.6. Does the agent establish a latent or persistent infection?	wtVSV can establish persistent infections in cell culture, due to the presence of specific mutations or through the activity of defective interfering (DI) particles	Persistent VSV infection has been established in Syrian Hamsters following intra-peritoneal (IP) injection of virus in the presence of DI particles. No evidence of virus persistence was seen in rodents inoculated IM with highly attenuated rVSV vectors	[63,68–70]
3.7. How does the wild type agent normally transmit?	In nature wtVSV is normally transmitted from biting insects to livestock and possibly rodents and other small mammals. Infected livestock may transmit to other animals by direct contact with vesicular lesions and by virus shed into feeding troughs	It is thought the major reservoir for VSV in nature is biting insects, as the virus can be passed vertically from adult to eggs. However, it appears that amplification to high titer in livestock is also important for longer term survival of the virus in nature, as continuous vertical transmission may result in diminishing virus titer (L. Rodriguez; personal communication)	[71–75]
3.8. What is known about the mechanisms of immunity to the wild type agent?	Immunity is acquired through a neutralizing humoral response to the virus G protein, which is located on the surface if the virus particle	CD8 T-cell responses may also contribute to VSV immunity, and have been mapped to the virus N protein in Balb/C mice; other T cell epitopes presumably are present in the remaining 4 major virus proteins	D. Cooper. Wyeth/Pfizer, unpublished data. [22,23]
3.9. Is there treatment required and readily available for the disease caused by the wild type agent?	Livestock are typically not treated with any therapeutic agent, and disease in humans is usually not severe enough to warrant any special treatment	Alpha and beta interferons have a potent anti-viral activity in cell culture; and demonstrated anti-viral activity *in vivo*	[76]

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Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors			
4. Characteristics of proposed vaccine vector	**Information**	**Comments/Concerns**	**Reference(s)**
4.1. What is the basis of attenuation/inactivation?	Attenuation of virulence is based on a reduction of viral replication and particle maturation efficiency	The actual attenuating mutations are a combination of N gene translocation and G gene/protein truncation which results in synergistic attenuation of virulence	[47,49,50]
4.2. What is the risk of reversion to virulence or recombination with wild type or other agents?	The risk is very low	The attenuating mutations comprise major alterations of the viral genome that cannot be directly reverted.	
4.3. Is the vector genetically stable during multiple passages?	Yes. To date, two genetically stable lots of clinical trial material (CTM) have been produced. All other vectors on a clinical pathway have also demonstrated genetic stability. However, upon prolonged passage in Vero cell culture a small number of point mutations accrued throughout the genome, which were associated with improved growth in Vero cells but not in BHK cells; presumably Vero cell specific adaptation (Wyeth; Unpublished observations)	Deletions, frame shifts and small insertions may also occur in the genes encoding antigens, but at much lower frequency than point mutations. These changes are typically fixed only when there is a strong selective advantage to the virus	[77]
4.4. What is known about the genetic stability during *in vivo* replication?	During natural outbreaks of infection with wtVSV in livestock there is often some genetic drift; possibly due to immune selection and/or generation of genetic bottlenecks	Genomic sequencing of attenuated vector(s) has not been performed on virus recovered from animals following IM inoculation. However the very limited extent of virus replication observed in the most permissive animal model (mouse), indicates little opportunity for selection of any genetic variants which may arise after vaccination	[63]
4.5. Will a replication competent agent be formed?	Yes. The current rVSV/HIV-1 clinical candidate is highly attenuated but replication competent	Propagation of the attenuated rVSV vector following IM inoculation is highly restricted (see 4.4)	
4.6. What is the potential for shedding and transmission?	Following IM inoculation of animals, there is no evidence of virus dissemination and shedding. No virus dissemination and shedding was observed following IM inoculation of rabbits during a GMP toxicology study. No virus dissemination in blood and no virus shedding in urine and saliva was detected in humans inoculated IM with an attenuated rVSV vector in a Phase 1 Clinical Trial (HVTN:090)	No virus shedding was detected in saliva, urine or blood in a second Phase I clinical trial evaluating a pDNA prime rVSV boost vaccination regimen (HVTN 087)	[63]
4.7. Will the agent survive in the environment?	The highly attenuated rVSV vectors would not survive in the environment for the following reasons: (1) The virus particles themselves are labile *ex vivo* (2) The virus is unable to cause vesicular lesions containing high titer progeny virus in livestock, thereby breaking a critical step in the virus lifecycle (3) The virus is not shed following IM inoculation	Replication of the attenuated rVSV/HIV-1 vaccine vector(s) in insects has not been studied.	[30,78]

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Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors			
4.8. Is there a non-human 'reservoir'?	Yes. The wtVSV reservoir is currently believed to be in biting insects such as sandflies	No known reservoir for the attenuated rVSV vector	
4.9. Is there any evidence for or against safety during pregnancy?	There is currently no evidence for or against safety during pregnancy in humans	There is no evidence that wtVSV can cause abortions in livestock following natural infection	
4.10. Can the vector accommodate multigenic inserts or will several vectors be required for multigenic vaccines?	rVSV vectors have been designed that accommodate multigenic inserts. (Profectus Biosciences; Unpublished data). Multiple vectors expressing multiple antigens can also be blended in a single vaccine formulation	The ability to accommodate multigenic inserts may depend on the size of the genes and any toxicity of the gene products on rVSV replication	[32,33,79]
4.11. What is known about the effect of pre-existing immunity on 'take', safety or efficacy in animal models?	Pre-existing VSV serotype specific immunity may reduce immunogenicity of corresponding serotype rVSV vectors; depending on the level of neutralizing antibody present in the blood. However, the seroprevalence of VSV is very low in the human population	Multiple different vesiculovirus serotypes exist. The different serotypes may be exploited to circumvent pre-existing immunity that might occur during vaccination prime-boost regimens	[80]
5. Manufacturing	**Information**	**Comments/Concerns**	**Reference(s)**
5.1. Describe the source (e.g. isolation, synthesis).	The rVSV vectors are recovered from a cDNA copy of the attenuated virus genome in a process known as "virus rescue". Genomic cDNA was prepared from highly attenuated laboratory adapted natural VSV isolates	The rescue process is performed under carefully controlled and documented conditions and reagents (Compliant Rescue) as a prelude to GMP manufacture	[81,82]
5.2. Describe the provenance of the vector including passage history and exposure to animal products. Describe the provenance of the Mudd-Summers strain and its derivation and rescue by Rose et al.	Following rescue from genomic cDNA the rVSV vector is plaque purified and passaged 10-15 times on Vero cell monolayers to generate research virus seed (RVS). The RVS is then amplified in a bioreactor to generate master virus seed (MVS). MVS is then used to inoculate fresh Vero cells in a second bioreactor run to produce clinical trials material (CTM). Exposure to animal products is highly restricted. Any animal products used must be from an approved region of the world and have an acceptable certificate of analysis	The original rVSV vector generated in the laboratory of Dr. John Rose (Yale) was a chimera, containing portions of the genome derived from the San Juan and Mudd Summer isolates of the Indiana serotype of VSV. Both VSV isolates were passaged multiple times on BHK cell monolayers before being used to generate the infectious cDNA clone described by Lawson et al. This rVSV vector was then further modified by the attenuation strategy described in Section 4.1 to generate a vector suitable for clinical evaluation	[81]
5.3. Can the vector be produced in an acceptable cell substrate?	Yes. The vector is rescued, amplified and manufactured on an approved Vero cell line	The Vero cell line has been extensively tested for the absence of mycoplasma and a large spectrum of other adventitious agents	
5.4. Describe the proposed production process.	See 5.2		
5.5. What are some Purity/ Potential contaminants?	The purification process has been validated and documented. One impurity identified are HIV-1 Gag virus like particles (VLP) composed of Gag protein expressed by rVSV. The Gag VLP is present in CTM at very low levels and does not influence the magnitude of Gag-specific immune responses	Another impurity is host cell DNA. The level of contaminating DNA is low (<50 ng/vaccine dose) and the bulk of contaminating Vero cell DNA is composed of small DNA fragments <500 bp in length, and is therefore not considered a significant safety issue	

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Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors			
5.6. Is there a large-scale manufacturing feasibility?	Yes	Vaccine has been manufactured at 10 L scale, but there is potential for scale up to 100 L, 1000 L or 10,000 L manufacturing runs	
5.7. Are there any IP issues and is there free use of the vector?	There are no IP issues for Profectus Biosciences, which has licensed the IP in the area of rVSV use as a vaccine vector for a range of human pathogens		
6. Toxicology and potency (Pharmacology)	**Information**	**Comments/Concerns**	**Reference(s)**
6.1. What is known about the replication, transmission and pathogenicity in animals?	The attenuated rVSV vaccine vector is not pathogenic in animals, even after direct injection of the brain. Following IM inoculation, replication of the attenuated rVSV vector is greatly reduced relative to wtVSV and is limited to the site of inoculation and the draining lymph node in murine models. The attenuated rVSV vector does not transmit after IM injection	No shedding of rVSV vectors has been detected in rabbits and humans inoculated IM with the Profectus Biosciences attenuated rVSV vectors. Vectors undergo very limited replication *in vivo* and are non pathogenic	[18,30]
6.2. For replicating vectors, has a comparative virulence and viral kinetic study been conducted in permissive and susceptible species? (yes/no) If not what species would be used for such a study? Is it feasible to conduct such a study?	Comparative virulence has been performed in a very sensitive mouse intra-cranial (IC) lethal dose-50 (LD_{50}) model. The mouse is a very permissive host for VSV, and kinetic (biodistribution) studies have been performed in mice following IM inoculation	The mouse is highly susceptible to VSV infection and replication	[50,63]
6.3. Does an animal model relevant to assess attenuation exist?	Yes. Both mouse and NHP models have been used to assess attenuation. The mouse model is the most sensitive of the two	The attenuated rVSV vaccine vectors tested in the clinic demonstrated an extremely low level of pathogenicity even when directly injected into the brain of mice and NHPs	[46,50]
6.4. Does an animal model for safety including immuno-compromised animals exist?	These studies have not yet been performed, but immuno-compromised animal models are available for testing		
6.5. Does an animal model for reproductive toxicity exist?	Not yet		
6.6. Does an animal model for immunogenicity and efficacy exist?	Yes. Murine and NHP immunogenicity models are in use, and SHIV challenge models have been used to assess protective efficacy of rVSV vectors in NHPs	rVSV vaccine vectors have demonstrated outstanding protective efficacy in animal models of disease for a range of human pathogens	[42,83,84]
6.7. What is known about biodistribution?	Biodistribution studies have been performed in mice and ferrets. Virus replication is restricted to the IM site of inoculation and the draining lymph node	No other major organs and tissues showed viral involvement; no virus could be detected in the blood or brain following IM inoculation. No virus can be detected in the blood, saliva and urine of humans following IM injection (HVTN 090 and HVTN 087)	[63] [18]
6.8. Have neurovirulence studies been conducted?	Yes. Extensive neurovirulence testing has been performed in mice and NHP	The attenuated rVSV vector developed by Profectus Biosciences has demonstrated safety in both the mouse and NHP NV models. The attenuated rVSV vector causes little more injury in the brain than inactivated virus. The most notable form of pathology is a mild, transient inflammatory response	[47,49,50]

Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors			
6.9. What is the evidence that the vector will generate a beneficial immune response with HIV or another disease in:	Evidence of beneficial immune responses to HIV-1 and other pathogens have been widely published		[83–89]
• Rodent?	Yes. Numerous publications		[42,86,87]
• Non-rodent?	Yes, rabbits.		[88]
• NHP?	Yes		[83,84]
• Human?	Yes		[18]
6.10. Have challenge or efficacy studies been conducted with:			
• HIV?	SHIV challenge studies have been performed in NHP		[83,84]
• Other diseases?	Yes. Challenge studies have been performed in rodents for a range of disease agents including influenza and respiratory syncytial virus; studies have also been performed in rabbits for papillomavirus		[85–89]
7. Previous Human Use	**Please type one of the following: Yes, No, Unknown, N/A (non-applicable)**	**Comments**	**Reference(s)**
7.1. Has the vector already been used for targeting the disease of vector origin?	No		
7.2. Is there information about the replication, transmission and pathogenicity of the vector in the following population? If so, what is known?			
• Healthy people?	Yes	The attenuated rVSV vector is not pathogenic in people and is not shed in saliva and urine following IM inoculation	[18]
• Immunocompromised?	Unknown		
• Neonates, infants, children?	Unknown		
• Pregnancy and in the unborn?	Unknown		
• Gene therapy experiments?	Unknown		
• Any other susceptible populations?	Unknown		
7.3. Is there any previous human experience with a similar vector including in HIV+ (safety and immunogenicity records)?	Yes.	Some forms of rVSV vectors are undergoing tests as oncolytic agents in humans, and as a vaccine for Ebola virus	Personal communication; Stephen Russell, Mayo Clinic [90–92]
7.4. Is there any previous human experience with present vector including in HIV+ (safety and immunogenicity records)?	No	The recently completed HVTN sponsored 090 clinical trial was the 1st testing of the current highly attenuated rVSVN4CT1 vector in humans; however, there is an ongoing clinical trial with this vector in HIV-1+ participants. No adverse events have been reported from this study to date	[18]
7.5. Is there information about the effect of pre-existing immunity on 'take', safety or efficacy in any human studies with this or different insert? If so, what is known?	Yes	It is anticipated that pre-existing immunity to VSV would reduce the frequency of "take" and vaccine efficacy, however it was possible to boost immune responses in people with a second dose of homologous rVSV (HVTN 090). Clinical studies with measles virus vectors indicate pre-existing immunity might not interfere significantly with take	[93]

(*continued on next page*)

Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors				
7.6. Are there other non-HIV vaccines using same vector? If so, list them and describe some of the public health considerations.	Yes. The same vector design is being used to generate a vaccine for Ebola virus. The same vector is also under pre-clinical testing for use as a vaccine for chikungunya virus and Venezuelan, Eastern and Western equine encephalitis viruses		The rVSV vector has not yet completed human clinical trials for diseases other than HIV at present; but clinical testing of an Ebola virus vaccine based on this rVSV vector is currently in progress. The direct public health considerations will remain similar to those of the rVSVHIV vaccine vector	[10]
8. Overall Risk Assessment	**Describe the toxicities**	**Please rate the risk as one of the following: none, minimal, low, moderate, high, or unknown**	**Comments**	**Reference(s)**
8.1. What is the potential for causing serious unwanted effects and toxicities in:				
• Healthy people?	Fever, myalgia	Minimal		[18]
• Immunocompromised?	Fever, myalgia	Unknown		
• Neonates, infants, children?	Fever, myalgia	Unknown		
• Pregnancy and in the unborn?	Fever, myalgia	Unknown		
• Other susceptible populations?	Unknown	Unknown		
8.2. What is the risk of neurotoxicity/neuroinvasion or cardiac effects?		Minimal		[49,50]
8.3. What is the potential for shedding and transmission in at risk groups?		Based on the outcome of animal toxicity studies and a Phase 1 clinical trial, rVSV shedding after IM inoculation is highly unlikely		[18]
8.4. What is the risk of adventitious agent (including TSE) contamination?		Minimal		
8.5. Can the vector be manufactured at scale in an acceptable process?	Yes			
8.6. Can virulence, attenuation and toxicity be adequately assessed in preclinical models?	Yes			
8.7. Rate the evidence that a beneficial response will be obtained in humans.		Moderate to high		[18,90]
9. Adverse Effect Assessment	**Describe the adverse effects**	**Please rate the risk as one of the following: none, minimal, low, moderate, high, or unknown**	**Comments**	**Reference(s)**
9.1. Describe the adverse effects observed				
• Mild local reactions	Redness	Minimal		[18]
• Mild systematic reactions	Low fever	Minimal		[18]
• Moderate local reactions	Irritation and swelling	Minimal		[18]
• Moderate systematic reactions	Fever and myalgia	Minimal		[18]

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Table 1 (*continued*)

Risk/Benefit Assessment for Vaccine Vectors				
• Severe local reactions	Pain and swelling	None		
• Severe systematic reactions	High fever, myalgia, weakness	None		
10. Administration Assessment	**Information**		**Comments/Concerns**	**Reference(s)**
10.1. What is the average Tissue Culture Infections Dose per milliliter (TCID/ml)?	10^7 plaque forming units (pfu)/mL		Value will be similar to TCID/mL	
10.2. What is the highest TCID/ml that can be used before cell toxicity?	Unknown		No Toxicity has been observed in mice and rabbits up to 10^8 pfu input	
10.3. Are different demographics affected differently?	Unknown		Very unlikely that different demographics will be differently affected	

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