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Immunomonitoring of human responses to the rVSV-ZEBOV Ebola vaccine

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The rVSV-ZEBOV vaccine is currently the only Ebola vaccine with demonstrated clinical efficacy in a ring-vaccination clinical trial. It has been shown to be reactogenic but immunogenic and safe in several Phase I clinical studies. However, its mechanisms of protection are unknown and available immunogenicity data are mostly limited to classical serological analysis; it is now of paramount importance to apply cutting-edge technologies, including transcriptomic and metabolomic analyses, and to perform integrative analyses with standard serology and clinical data to comprehensively profile the rVSV-ZEBOV immune signature.

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Need for an Ebola vaccine

Ebola Virus Disease (EVD) has occurred in numerous sporadic outbreaks since its identification. In 2014, West Africa experienced the largest outbreak of Ebola Virus Disease (EVD) in history, with six countries affected: Guinea, Liberia, Nigeria, Senegal, Mali and Sierra Leone; with over 28 652 confirmed or probable cases and more than 11 325 deaths [URL://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/; [1,2]. The extremely high mortality and the risks of exportation have triggered a high level of global concern. Furthermore, Ebola viruses may become endemic in countries with poor health care infrastructures, thus representing a significant long-term threat.

Currently, no effective therapies or licensed vaccines exist for Ebola virus and for any member of the Filoviridae family. The availability of effective Ebola vaccines could be of critical importance for preventing the disease in high risk areas, using ring vaccination strategies, and would maximize safety for front line workers at greatest risk during outbreaks [3]. An immunization capable of rapidly eliciting sustained protective immunity in most recipients with a single dose would be highly desirable.

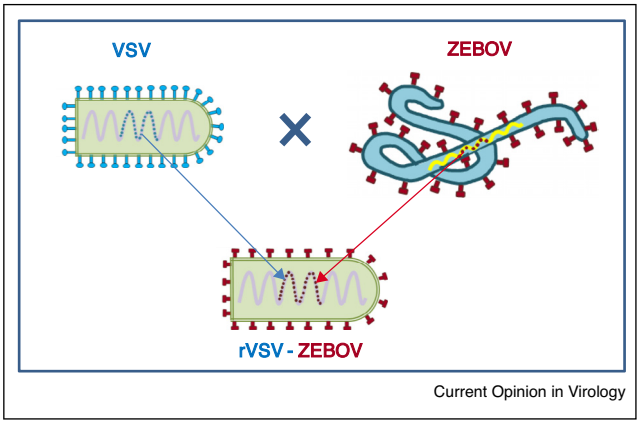
The 2014 EVD outbreak promoted a strong unprecedented effort for the development of Ebola vaccines and several vaccine candidates were advanced to the clinic including: (i) the ChAd3-EBO non-replicating vaccine vector based on the chimpanzee adenovirus type 3 (ChAd3) [4,5]; (ii) the heterologous prime-boost Ebola vaccine regimen based on recombinant adenovirus type 26 vector (Ad26.ZEBOV) priming followed by boosting with modified vaccinia Ankara vector (MVA-BN-Filo©) [6]; (iii) the adenovirus 5-vectored Ebola vaccine [7–9], (iv) the DNA Ebola vaccine [10,11] and (v) the recombinant Vesicular Stomatitis Virus (VSV) vector-based Ebola vaccine (rVSV-ZEBOV), the most advanced candidate to have shown immunogenicity, safety and protective efficacy in humans [12••,13••,14••,15••,16••] on which this review focuses.

The rVSV-ZEBOV vaccine candidate

The vector is a single recombinant VSV wild type isolate in which the VSV envelope glycoprotein was replaced with the Zaire strain Ebola virus (ZEBOV) glycoprotein (GP), giving rise to rVSVΔG-ZEBOV-GP (rVSV-ZEBOV) (Figure 1) [17,18]. This Ebola vaccine candidate was developed by Public Health Canada (BPSC1001), licensed to NewLink Genetics and subsequently transferred to Merck (renamed as V920). The characteristics of rVSV-based vectors are reviewed in Ref. [17]. Previous experience showed that rVSV lacks the potential for reassorting with viruses and cannot integrate into host cell DNA. The *in vivo* replication of rVSV-ZEBOV is significantly attenuated by the exchange of the rVSV glycoprotein for the EBOV-GP. The VSV-ZEBOV vaccine was identified by the international consultation led by WHO in September 2014 as one of the only two Ebola vaccine candidates with 100% demonstrated protection in non-human primates (NHP) and clinical grade material ready for clinical testing [3].

Protection against EVD was first reported in nonhuman primates (NHP) with a single intramuscular (IM) injection of rVSV-ZEBOV [19,20]. Notably, vaccine vector

Figure 1



Schematic representation of rVSV-ZEBOV. The rVSV-ZEBOV vaccine is a recombinant virus in which the VSV envelope glycoprotein (in blue) is replaced with the Zaire strain Ebola virus (ZEBOV) glycoprotein (in red) giving rise to the chimeric virus rVSVΔG-ZEBOV-GP (rVSV-ZEBOV).

shedding was not detectable in NHP and none of the animals developed overt fever or other detectable adverse events [19,21]. rVSV-ZEBOV induced humoral and cellular immune responses in all vaccinated monkeys and no evidence of Ebola virus replication was detected after intravenous challenge with a high lethal dose of ZEBOV. Complete and partial protection was even achieved with a single injection seven or three days before challenge, respectively, showing that VSV-ZEBOV may rapidly confer protection further supporting its use for rapid responses during outbreaks [22]. Safety in immunocompromised hosts, an important consideration given the potential presence of HIV infected individuals in the target vaccine population, was evaluated in a few rhesus macaques infected with simian-human immunodeficiency virus (SHIV), none of the infected animals showed evidence of illness following rVSV-ZEBOV immunization and four out of six were protected from EBOV challenge [23]. The rVSV-ZEBOV vaccine also showed an efficacy of 33–67% following a 24 hours post-exposure injection of Resus macaques infected with Ebola virus Makona [24]. The potential for protection against newly emerging, phylogenetically related strains was assessed by immunizing macaques with rVSV-ZEBOV prior to challenge with Bundibugyo ebolavirus (BEBOV), a newly emerged EBOV species [25]. A single vaccination with rVSV-ZEBOV provided significant cross-protection (75% survival), suggesting that monovalent rVSV-based vaccines might be also useful against newly emerging species [23].

The rVSV-ZEBOV vaccine was tested in several clinical trials (Table 1) in over 1000 subjects, showing high immunogenicity, safety and protective efficacy [12••,13••,14••,15••,16••]. Phase I VEBCON clinical trials have been organized in Africa and Europe as investigator-driven and investigator-sponsored trials under the coordination of WHO, using vaccine vials donated to WHO by Public Health Canada and financially supported by a grant of the Wellcome Trust Foundation to the WHO. Additional funds were provided by the Bill and Melinda Gates Foundation and the German Center for Infection Research. The first results of the safety and immunogenicity of rVSV-ZEBOV were reported only a few months after the initiation of the first clinical trials [12••,13••]. At high doses, ranging between 3×10^6 and 5×10^8 PFU, mild-to-moderate reactogenicity (fever, myalgia, chills, fatigue, headaches, *etc.*) affected most subjects. Symptoms occurred early (onset day 1 and 2) and were transient, associated to viremia and haematological changes reflecting vaccine replication [13••]. The rVSV-ZEBOV vaccine generated glycoprotein-binding antibodies in almost all participants at any dose, showing its high immunogenicity in humans. Unexpectedly, oligoarthritis was identified in the second week after vaccination with $\geq 10^7$ PFU in 11/51 (22%) of Geneva clinical trial subjects. It lasted an average of 8 days, occasionally associated with maculopapular or vesicular dermatitis. The identification of rVSV-ZEBOV in synovial fluid and skin vesicles confirmed viral dissemination and replication in peripheral tissues [14••]. Only two similar cases of arthritis were observed at other VEBCON trial sites, suggesting an influence of the vaccine dose, host factors, and/or reporting and investigative approaches.

After a safety-driven study hold, the Geneva randomized clinical trial was restarted with a lower dose of 3×10^5 PFU and the Gabon/German trials were conducted at the same or lower doses, providing the opportunity to assess the relative influence of vaccine dose on vaccine safety and immunogenicity in the same populations. Reducing the vaccine dose markedly reduced acute reactogenicity, but did not avoid arthritis, dermatitis or cutaneous vasculitis, and limited the magnitude of antibody responses [14••]. No vaccine-associated severe adverse events were reported in any of the rVSV-ZEBOV phase 1 trials. Hence, the vaccine was selected for use at a dose of 2×10^7 PFU in further phase 2/3 trials sponsored by WHO (Guinea), the U.S. Centers for Disease Control and Prevention (CDC) (Sierra Leone) and the U.S. National Institute of Health (NIH) (Liberia), along with the National Health Authorities of the host countries.

Protective efficacy was demonstrated in an open-label, cluster-randomised ring vaccination trial in the communities of Conakry, Guinea, and Sierra Leone [15••,16••]. The analysis included a total population of 11 841 individuals, assigned to 117 clusters (rings) vaccinated either immediately or after a 21-day delay. rVSV-ZEBOV showed up to 100% efficacy, with no cases of EVD as of 10 days after immunization, compared to 23 cases in yet unvaccinated subjects [16••]. In fact, new cases of EVD

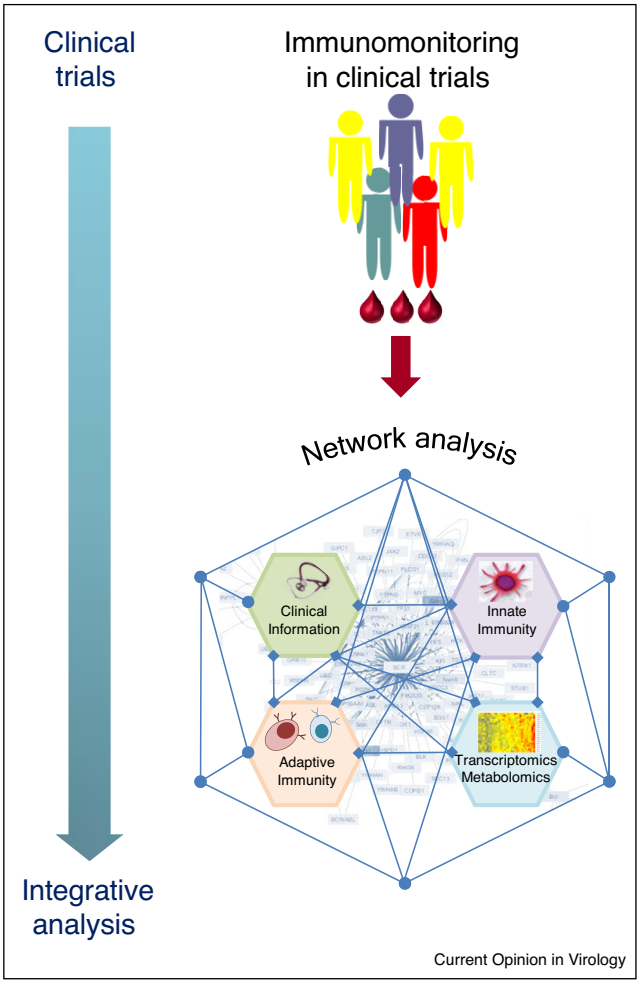
Table 1

Clinical trials with the rVSV-ZEBOV vaccine

Phase	Dose (pfu)	Subjects n.	Age (years)	Status	Sponsor	Country	Clinical trial n.	Ref.
1 – Safety, and immunogenicity	2.5×10^4 2.5×10^5 2.0×10^6	38	18–65	Completed	Profectus Bioscience Inc.	USA	NCT02718469[a]	
1 – Safety, and immunogenicity	3.0×10^6 2.0×10^7 1.0×10^8	39	18–65	Completed	Merck Sharp & Dohme Corp	USA	NCT02280408[a]	[13••]
1 – Safety, tolerability and immunogenicity	3.0×10^5 3.0×10^6 2.0×10^7	30	18–55	Completed	University of Hamburg-Eppendorf	Germany	NCT02283099	[12••]
1 – Safety and immunogenicity	3.0×10^3 3.0×10^4 3.0×10^5 3.0×10^6 9.0×10^6 2.0×10^7 1.0×10^8	512	18–60	Completed	Merck Sharp & Dohme Corp.	USA	NCT02314923	
1 – Safety, tolerability and immunogenicity	1.0×10^5 5.0×10^5 3.0×10^6	40	18–65	Completed	Dalhousie University	Canada	NCT02374385	
1 – Safety, tolerability and immunogenicity	3.0×10^6 1.0×10^7	40	18–55	Ongoing	University of Oxford	Kenya	NCT02296983	[12••]
1 – Safety and immunogenicity	3.0×10^3 3.0×10^4 3.0×10^5 3.0×10^6 2.0×10^7	155	6–50	Ongoing	University of Tuebingen	Gabon	PACTR201411000919191	[12••]
1 – Immune durability	3.0×10^5 1.0×10^7 5.0×10^7	100	18–68	Ongoing	University Hospital Geneva	Switzerland	NCT02933931	
1 – Safety and immunogenicity	3.0×10^6 2.0×10^7 1.0×10^8	39	18–50	Completed	Merck Sharp & Dohme Corp.	USA	NCT02269423	[13••]
1/2 – Safety and tolerability	3.0×10^5 1.0×10^{7b} 5.0×10^{7b}	115	18–65	Completed	University Hospital Geneva	Switzerland	NCT02287480	[12••,14••]
2 – Immunogenicity and durability	2.0×10^7	300	>18	Ongoing	NIAID	USA	NCT02788227	
2 – Safety and efficacy	2.0×10^7	900	>18	Ongoing	NIAID	Liberia	NCT02344407	
2/3 – Safety immunogenicity	2.0×10^7	8000	>18	Ongoing	CDC	Sierra Leone	NCT02378753	
3 – Safety, immunogenicity and efficacy	2.0×10^7	8851	6–90	Completed	WHO	Guinea (Conakry)	PACTR201503001057193	[15••,16••]
3 – Safety, immunogenicity and efficacy	3 consistency lots and a high-dose lot	1198	18–65	Ongoing	Merck Sharp & Dohme	USA	NCT02503202	

[a] Prime-boost schedule.
[b] Interrupted on January 2015.

Figure 2



Integrative immunological analysis of rVSV-ZEBOV. Immunomonitoring in clinical trials is conducted to identify molecular signatures of the rVSV-ZEBOV vaccine integrating standard analysis of innate and adaptive immune responses and clinical data with transcriptomic and matabolomic analysis.

were not diagnosed from six days after vaccination, indicating the rapid onset of protective immune responses considering that exposure may have occurred prior to immunization [15••]. Adverse effects, mostly mild and self-limiting (headache, fatigue and muscle pain), were reported in more than half of vaccinees. These results demonstrated the feasibility of using rVSV-ZEBOV in a ring-vaccination design to help control outbreaks. A Merck-sponsored phase III clinical trial is ongoing to evaluate the safety and immunogenicity of three different lots of rVSV-ZEBOV in about 1200 healthy adults. The primary purpose of the study is to demonstrate consistency in the immune responses of participants receiving three separate lots of V920 through 28 days post-vaccination. A subset of participants will continue to be monitored through 24 months post-vaccination for the evaluation of safety, and durability of immune response (NCT02503202).

Yet, many questions are still open. They mainly relate to the safety and immunogenicity of rVSV-ZEBOV in vulnerable populations such as young children or pregnant women and to the duration of protection. In the absence of current outbreaks, these may only be assessed by immunomonitoring.

Immunomonitoring of rVSV-ZEBOV

Conventional serology and cellular immunology provide significant insights into vaccine induced protective immunity. However the integration of multiple layers of information derived from the integration of clinical and state-of-the-art immunological read outs with distinct 'Omics' analyses, such as transcriptomics and metabolomics, may provide a better understanding of the complex mechanisms of vaccine-induced protective reactogenicity and immunity (Figure 2). This may help in identifying early signatures/biomarkers predictive of magnitude, quality and duration of vaccine-induced adaptive immune responses as well as surrogate markers of vaccine induced adverse events. Recently, systems biology approaches have been employed to pinpoint signatures of immunogenicity for few human vaccines [26]. Using the latest systems biology techniques and models (including integrated and validated transcriptomics and metabolomics analyses) to dissect the complexity of the interactions between the various components of the human immune system in response to vaccination is of critical importance to fully decipher the immunological signature of rVSV-ZEBOV [27,28].

Serological analyses conducted in Phase 1 clinical trials using escalating vaccine doses have shown that EBOV–glycoprotein (GP)–specific IgG antibody responses were detected in almost all participants, with significantly higher titres of EBOV neutralizing antibodies at higher vaccine doses [14••]. The importance of a predominantly anti-GP IgM response for EBOV neutralization was revealed as well as an independent evolution of antibody immune responses – in terms of antibody epitope repertoire diversity, affinity maturation, durability and isotype switching – after vaccination with rVSV-ZEBOV in three dose groups (3 million, 20 million and 100 million PFUs) [29•]. A strong correlation between *in vitro* EBOV neutralization and serum GP-binding antibody titres was observed. Interestingly, a second dose did not boost antibody or virus neutralization titers and elicited limited antibody affinity maturation [27]. It has also been recently shown that ZEBOV-specific circulating follicular T helper cells (cTfh) correlate with antibody titers and with the Tfh17 subset [30].

In a recent prospective derivation and validation cohort study nested within the phase I randomized, placebo-

controlled (Geneva, Switzerland) and dose-escalation (Lambaréné, Gabon) trials, we observed an early (day 1) dose-dependent plasma signature of the safety and immunogenicity of the rVSV-Ebola vaccine. These data highlight that monocytes play a critical role in rVSV-ZEBOV-linked reactogenicity and adverse events, including arthritis [31••]. Preliminary analysis of whole-blood transcriptomic data, obtained by targeted transcriptome sequencing on Ion Proton platform, revealed an activation of genes involved in innate immune responses, which starts at day 1 after vaccination and lasts until day 7 (manuscript in preparation).

Results obtained from the rVSV-ZEBOV clinical trials so far have shown that there are differences among volunteers receiving different doses of the vaccine as pertain to the magnitude of the antibody response as well as the magnitude and duration of adverse events. Hence, this stratified response to the vaccine offers a unique opportunity for biomarker discovery. The rVSV-ZEBOV phase I trials performed in Geneva, Lambaréné and Kilifi have generated a unique set of immunological and vaccine safety observations, the biological bases of which are now being studied through thousands of collected biological samples. A significant step beyond the state-of-the-art is now required, through the harmonisation and in depth integrated analyses of data generated by all different clinical and immunological/molecular read outs. This includes safety, immunogenicity, innate and adaptive immunity, immunological memory, transcriptomics and metabolomics (Figure 2). This will ensure gaining maximum leverage of the Phase I Ebola vaccine trials, through a comprehensive characterization of the immune responses induced by rVSV-ZEBOV and ensuring that all information resulted from clinical studies is fully exploited and shared. Indeed, the field protective efficacy trials did not include harvesting samples for immunomonitoring [15••,16••].

Joining efforts to profile immune and molecular signatures of rVSV-ZEBOV

The VSV-EBOVAC project (www.vsv-ebovac.eu) supported by Innovative Medicines Initiative 2 Joint Undertaking (IMI2 JU, http://www.imi.europa.eu/), was launched to comprehensively characterize the immune and molecular signatures induced in humans by the rVSV-ZEBOV vaccine [32••]. VSV-EBOVAC seeks to carry out in-depth transcriptomics and metabolomics analyses of blood samples obtained at different time points following immunization with rVSV-ZEBOV in Switzerland, Kenya and Gabon, harnessing state-of-the-art and cutting edge technologies to carry out systems analysis of human innate and adaptive immune responses to rVSV-ZEBOV. Transcriptomic response is assessed in the RNA extracted from blood stored in PAXgene tubes and RNA sequencing is performed using the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit (Thermo Fisher) on an Ion Proton instrument. This protocol allows the simultaneous quantification of transcripts from 20802 human genes [28]. Gene expression data are correlated with clinical and immunologic data and analyzed using the blood transcription modules identified in previous vaccinology studies [27]. Metabolomic analyses are performed on plasma samples using the qTOF LC/MS system (Agilent) which combines accuracy and sensitivity. Targeted metabolomics using ISQ GC–MS platform (Thermo Fisher) is carried out for absolute quantification of a limited number of key metabolites. Integrative data analysis using a systems biology approach are being employed to pinpoint molecular patterns and pathways. The underlying premise is that results obtained from such integrated omics approach, combined with clinical and immunological read outs, could lay a foundation for the discovery of molecular biomarkers of rVSV-ZEBOV vaccine safety and immunogenicity.

Synergy and complementarity with other Ebola vaccine projects as well as with other EU projects active in the field of systems vaccinology such as the High Impact FP7 Project on Advanced Immunization Technologies (ADITEC, www.aditecproject.eu), aiming to accelerate the development of novel, powerful immunisation technologies for next-generation vaccines [33], are also promoted by VSV-EBOVAC. It is expected that the results obtained from this joint efforts will enhance and accelerate the development of rVSV-ZEBOV as a safe and efficacious vaccine to counter Ebola infection in humans.

Concluding remarks

rVSV-ZEBOV is currently the only Ebola vaccine with demonstrated efficacy in a ring vaccination clinical trial as well as excellent immunogenicity and safety. Current immunogenicity data are limited to adult responses and the use of serology, showing the important role of antibodies in protection with emerging evidences indicating the key involvement of innate immune responses in the shaping of both immunogenicity and safety. Applying cutting-edge technologies including transcriptomic and metabolomic analyses, and integrating with clinical and serological data is needed to fully decipher the rVSV-ZEBOV vaccine immune signature.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dixon MG, Schafer IJ: **Ebola viral disease outbreak—West Africa**. *MMWR Morb. Mortal. Wkly. Rep.* 2014, **63**:548-551.

2. Van Kerkhove MD, Bento AI, Mills HL, Ferguson NM, Donnelly CA: **A review of epidemiological parameters from Ebola outbreaks to inform early public health decision-making**. *Sci. Data* 2015, **2**:150019.

3. Kanapathipillai R, Henao Restrepo AM, Fast P, Wood D, Dye C, Kieny M-P, Moorthy V: **Ebola vaccine — an urgent international priority**. *N. Engl. J. Med.* 2014, **371**:2249-2251.

4. Ledgerwood JE, DeZure AD, Stanley Da, Coates EE, Novik L, Enama ME, Berkowitz NM, Hu Z, Joshi G, Ploquin A, Sitar S *et al.*: **Chimpanzee adenovirus vector Ebola vaccine — preliminary report**. *N. Engl. J. Med.* 2017, **9**:928-938 http://dx.doi.org/10.1056/NEJMoa1410863.

5. Rampling T, Ewer K, Bowyer G, Wright D, Imoukhuede EB, Payne R, Hartnell F, Gibani M, Bliss C, Minhinnick A *et al.*: **A monovalent chimpanzee adenovirus Ebola vaccine boosted with MVA**. *N. Engl. J. Med.* 2015, **374**:1635-1646 http://dx.doi.org/10.1056/NEJMoa1411627.

6. Geisbert TW, Bailey M, Hensley L, Asiedu C, Geisbert J, Stanley D, Honko AN, Johnson J: **Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge**. *J. Virol.* 2011, **85**:4222-4233.

7. Li J-X, Hou L-H, Meng F-Y, Wu S-P, Hu Y-M, Liang Q, Chu K, Zhang Z, Xu J-J, Tang R *et al.*: **Immunity duration of a recombinant adenovirus type-5 vector-based Ebola vaccine and a homologous prime-boost immunisation in healthy adults in China: final report of a randomised, double-blind, placebo-controlled, phase 1 trial**. *Lancet Glob. Health* 2016, **5**:324-334 http://dx.doi.org/10.1016/S2214-109X(16)30367-9.

8. Zhu F-C, Wurie AH, Hou L-H, Liang Q, Li Y-H, Russell JBW, Wu S-P, Li J-X, Hu Y-M, Guo Q *et al.*: **Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial**. *Lancet* 2016, **389**:621-628 http://dx.doi.org/10.1016/s0140-6736(16)32617-4.

9. Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamshchikov G, Mulangu S, Hu Z, Andrews CA, Sheets RA *et al.*: **A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults**. *Vaccine* 2010, **29**:304-313.

10. Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB, Costner P, Sitar S, Glover D, Hu Z *et al.*: **Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: A phase 1b, randomised, double-blind, placebo-controlled clinical trial**. *Lancet* 2015, **385**:1545-1554.

11. Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS, Sitar S, Plummer S, Mulangu S, Bailer RT *et al.*: **Safety and immunogenicity of DNA vaccines encoding ebolavirus and marburgvirus wild-type glycoproteins in a phase i clinical trial**. *J. Infect. Dis.* 2015, **211**:549-557.

12. •• Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, Yerly S, Dayer JJ-A, Kraehling V, Kasonta R *et al.*: **Phase 1 trials of rVSV Ebola vaccine in Africa and Europe**. *N. Engl. J. Med.* 2015, **347**:1647-1660.
Preliminary report of three open-label, dose-escalation phase 1 trials and one randomized, double-blind, controlled phase 1 trial to assess safety and immunogenicity of rVSV-ZEBOV at various doses in 158 healthy adults in Europe and Africa. After a single dose rVSVZEBOV resulted reactogenic but immunogenic and safe.

13. •• Regules J, Beigel JH, Paolino KM, Voell J, Castellano AR, Hu Z, Muñoz P, Moon JE, Ruck RC, Bennett JW *et al.*: **A recombinant vesicular stomatitis virus Ebola vaccine**. *N. Engl. J. Med.* 2017, **26**:330-341 376.
Report of two phase 1, placebo-controlled, double-blind, dose-escalation trials of rVSV-ZEBOV in 78 adults showing that the vaccine elicits anti-Ebola antibody responses and transient rVSV viremia.

14. •• Huttner A, Dayer J, Yerly S, Combescure C, Auderset F, Desmeules J, Eickmann M, Finckh A: **The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial**. *Lancet Infect. Dis.* 2015, **15**:1156-1166.
Report on phase 1/2, dose-finding, trial in healthy adults conducted in Switzerland, showing that reducing the dose of rVSV-ZEBOV improves lowered antibody responses and did not prevent vaccine-induced reactogenicity (arthritis, dermatitis, or vasculitis.

15. •• Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, Carroll MW, Doumbia M, Draguez B, Duraffour S *et al.*: **Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial**. *Lancet* 2015, **386**:857-866.
Interim report on efficacy and effectiveness of a single dose (2×10^7) of rVSV-ZEBOV in ring vaccination in Guinea.

16. •• Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, Carroll MW, Dean NE, Diatta I, Doumbia M *et al.*: **Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!)**. *Lancet* 2016, **386**:857-866 http://dx.doi.org/10.1016/S0140-6736(16)32621-6.
Complete report on efficacy and effectiveness of rVSV-ZEBOV in ring vaccination in Guinea.

17. Garbutt M, Liebscher R, Wahl-Jensen V, Jones S, Möller P, Wagner R, Volchkov V, Klenk H-D, Feldmann H, Ströher U: **Properties of replication-competent vesicular stomatitis virus vectors expressing glycoproteins of filoviruses and arenaviruses**. *J. Virol.* 2004, **78**:5458-5465.

18. Yang ZY, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ: **Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury**. *Nat. Med.* 2000, **6**:886-889.

19. Jones SM, Feldmann H, Ströher U, Geisbert JB, Fernando L, Grolla A, Klenk H-D, Sullivan NJ, Volchkov VE, Fritz EA *et al.*: **Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses**. *Nat. Med.* 2005, **11**:786-790.

20. Matassov D, Marzi A, Latham T, Xu R, Ota-Setlik A, Feldmann F, Geisbert JB, Mire CE, Hamm S, Nowak B *et al.*: **Vaccination with a highly attenuated recombinant vesicular stomatitis virus vector protects against challenge with a lethal dose of Ebola virus**. *J. Infect. Dis.* 2015, **212**:S443-S451.

21. Mire CE, Miller AD, Carville A, Westmoreland SV, Geisbert JB, Mansfield KG, Feldmann H, Hensley LE, Geisbert TW: **Recombinant vesicular stomatitis virus vaccine vectors expressing filovirus glycoproteins lack neurovirulence in nonhuman primates**. *PLoS Negl. Trop. Dis.* 2012, **6**:e1567.

22. Marzi A, Robertson SJ, Haddock E, Feldmann F, Hanley PW, Scott DP, Strong JE, Kobinger G, Best SM, Feldmann H: **Ebola vaccine. VSV-EBOV rapidly protects macaques against infection with the 2014/15 Ebola virus outbreak strain**. *Science* 2015, **349**:739-742.

23. Geisbert TW, Daddario-DiCaprio KM, Lewis MG, Geisbert JB, Grolla A, Leung A, Paragas J, Matthias L, Smith MA, Jones SM *et al.*: **Vesicular stomatitis virus-based Ebola vaccine is well-tolerated and protects immunocompromised nonhuman primates**. *PLoS Pathog.* 2008, **4**.

24. Marzi A, Hanley PW, Haddock E, Martellaro C, Kobinger G, Feldmann H: **Efficacy of vesicular stomatitis virus-Ebola virus postexposure treatment in Rhesus macaques infected with Ebola virus Makona**. *J. Infect. Dis.* 2016, **214**:S360-S366.

25. Mire CE, Geisbert JB, Marzi A, Agans KN, Feldmann H, Geisbert TW: **Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo Ebolavirus**. *PLoS Negl. Trop. Dis.* 2013, **7**.

26. Hagan T, Nakaya HI, Subramaniam S, Pulendran B: **Systems vaccinology: enabling rational vaccine design with systems biological approaches**. *Vaccine* 2015, **33**:5294-5301.

27. Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, Schmidt DS, Johnson SE, Milton A, Rajam G *et al.*: **Molecular signatures of antibody responses derived from a systems biology study of five human vaccines**. *Nat. Immunol.* 2014, **15**:195-204.

28. Li W, Turner A, Aggarwal P, Matter A, Storvick E, Arnett DK, Broeckel U: **Comprehensive evaluation of AmpliSeq transcriptome, a novel targeted whole transcriptome RNA sequencing methodology for global gene expression analysis**. *BMC Genom.* 2015, **16**:1069.

29. • Khurana S, Fuentes S, Coyle EM, Ravichandran S, Davey RT, Beigel JH: **Human antibody repertoire after VSV-Ebola vaccination identifies novel targets and virus-neutralizing IgM antibodies**. *Nat. Med.* 2016, **22**:1439-1447 http://dx.doi.org/10.1038/nm.4201.
Study elucidating the human antibody repertoire after administration of rVSV-ZEBOV and showing a predominat IgM response with a strong virus neutralization activity. A second vaccination did not boost antibody or virus neutralization titers, and induced only minimal antibody affinity maturation.

30. Farooq F, Beck K, Paolino KM, Phillips R, Waters NC, Regules JA, Bergmann-Leitner ES: **Circulating follicular T helper cells and cytokine profile in humans following vaccination with the rVSV-ZEBOV Ebola vaccine**. *Sci. Rep.* 2016, **6**:27944 http://dx.doi.org/10.1038/srep27944.

31. •• Huttner A *et al.*: **A dose-dependent plasma signature of the safety and immunogenicity of the rVSV-Ebola vaccine in Europe and Africa**. *Sci. Transl. Med.* 2017, **9** http://dx.doi.org/10.1126/scitranslmed.aaj1701.

32. •• Medaglini D, Harandi AM, Ottenhoff THM, Siegrist C-A, VSV-Ebovac Consortium: **Ebola vaccine R&D: filling the knowledge gaps**. *Sci. Transl. Med.* 2015, **7**:317ps24.
Description of system biology approach of the VSV-EBOVAC project to decipher the rVSV-ZEBOV vaccine immune signature.

33. Rappuoli R, Medaglini D: **ADITEC: joining forces for next-generation vaccines**. *Sci. Transl. Med.* 2012, **4** 128cm4–128cm4.