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Review

Correlates of vaccine-induced protective immunity against Ebola virus disease

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ABSTRACT

Ebola virus disease is a deadly infection which occurs in sporadic outbreaks. Several vaccine candidates have been developed. The most advanced candidate is the recombinant VSVΔG-ZEBOV-GP vaccine, in which the Vesicular Stomatitis Virus (VSV) envelope glycoprotein is replaced by the Zaire strain Ebola virus (ZEBOV) glycoprotein (GP). This vaccine demonstrated 100% protection in a ring vaccination trial performed in Guinea in 2015, was granted "Breakthrough Therapy Designation" by the FDA and PRIority Medicines (PRIME), and is currently (June 2018) used to support outbreak control in Democratic Republic of Congo. rVSVΔG-ZEBOV-GP elicits a strong and durable antibody response in most vaccinees. This sustained Ebola GP-specific antibody response correlates with an early activation of innate immunity, especially of monocytes and of type-I interferon induced genes. Despite significant progress in the characterization of vaccine-induced immunity, human correlates of protection against Ebolavirus infection have not yet been fully established. A systems biology approach, integrating clinical, immunological, transcriptomic and metabolomic data from pre-clinical and clinical vaccine studies, together with data from disease survivors, will be instrumental to identify Ebola vaccine correlates of protection. The information generated for the rVSVΔG-ZEBOV-GP vaccine may also help identify the correlates of protection of the other Ebola vaccine candidates.

1. Ebolavirus disease recent outbreaks

Ebolavirus (EBOV) is a member of the family *Filoviridae* and the causative agent of severe and often fatal hemorrhagic fever in humans and non-human primates (NHPs). *Ebolavirus* disease (EVD) has occurred in numerous sporadic outbreaks since it was discovered in 1976. Case fatality rates of up to 90% have been reported [1–4]. Although asymptomatic/mild cases occur and largely remain unreported, EVD is a severe disease. During the 2014 EVD outbreak, over 28.000 cases and 11.000 deaths have been reported in six West African countries (Guinea, Liberia, Nigeria, Senegal, Mali and Sierra Leone) (www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/) [5,6]. On May 2018, an outbreak of EVD appeared in Democratic Republic of Congo (DRC); this was the ninth outbreak in DRC during the last four decades, the previous one dating back only to May 2017. As of July 3, 2018, a total of 55 cases and 29 deaths are known/suspected, including 38 laboratory confirmed, 15 probable and 2 suspected cases (WHO EBOLA VIRUS DISEASE Democratic Republic of Congo External Situation Report http://www.who.int/ebola/situation-reports/drc-2018/en/) [7]. Ebola virus may become endemic, representing a significant long-term threat in countries with poor health care infrastructures. During severe outbreaks, those in close contact with infected victims are at greatest risk; these include healthcare-, government- and non-government- aid workers. The scale and potential threats of these outbreaks has led to extraordinary efforts and measures from the World Health Organization (WHO), Médecins sans Frontières (MSF) and other institutions to effectively contain the spread of disease and treat affected patients. WHO and local Ministries of Health have set as priorities the strengthening of surveillance and contact tracing, laboratory capacity, infection prevention and control, case management, community engagement, safe and dignified burials and vaccination (http://www.who.int/csr/don/23-may-2018-ebola-drc/en/).

Currently, no effective therapies or licensed vaccines exist for EVD or any other member of the Filoviridae family of viruses. The most advanced Ebola vaccine is the rVSVΔG-ZEBOV-GP vaccine. It is based on recombinant Vesicular Stomatitis Virus (VSV) vector in which the

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VSV envelope glycoprotein was replaced with the Zaire strain Ebola virus (ZEBOV) glycoprotein (GP) [8,9]. The short-term protective efficacy of the rVSVΔG-ZEBOV-GP vaccine was demonstrated in an open-label, cluster-randomized ring vaccination trial in the communities of Conakry, Guinea, and Sierra Leone [10,11] conducted at the tail of the 2014/2015 West Africa epidemic. This vaccine has been granted Breakthrough Therapy Designation by the Food and Drug Administration (FDA) and PRIority Medicines (PRIME) status from the European Medicines Agency (EMA) [Merck Press Release, 25 Jul 2016], which afford acceleration of development and approval processes for products indicated for serious and unmet medical needs. It is nevertheless not yet licensed, which limits its clinical use under the Expanded Access Framework – a compassionate use program that aims to provide access to medical products that have not yet received regulatory approval. During the ongoing 2018 DRC outbreak, MSF and WHO are using again the rVSVΔG-ZEBOV-GP vaccine [12]. MSF is running an open-label single group trial to provide additional information on vaccine safety and efficacy, planning to include 500 people. WHO has started to use rVSVΔG-ZEBOV-GP in the DRC under the Expanded Access Framework. Unfortunately, integrating research components to advance the knowledge of vaccine efficacy and characterise its correlates of protection proves challenging under this scheme and in emergency settings.

2. Ebola correlates of protection

Correlates of protection (CoP) are measurable markers that are statistically associated with vaccine efficacy. According to Plotkin and Gilbert [13], these can be divided in mechanistic (mCoP) or non-mechanistic (nCoP) depending on whether they are a causal effector of protection or not. Vaccine efficacy can be measured against different clinical endpoints, e.g. against infection, infectiousness, disease or death.

Known vaccine CoP (both mCoP and nCoP) are almost invariably antibodies [14], including neutralizing or opsonizing antibodies as mCoP. Nevertheless, antibody responses require collaboration from cellular responses to be elicited and protection may require a combination of innate, antibody and T cell responses to be effective.

For example, CoP after anti-pneumococcal vaccination are complex: anti-capsular opsonizing antibodies are the mCoP, but the ELISA antibody concentration considered as protective varies depending on the capsular serotype and the clinical readout. The concentration required to prevent carriage is higher than that required to protect from mucosal diseases (acute otitis media, sinusitis), which is in turn higher that the protective antibody concentration against invasive pneumococcal disease [15–17]. Moreover, animal studies employing monoclonal antibodies suggest that non-opsonic antibodies could also have a role in preventing carriage and invasion [18]. As another example, the correlate of protection from infection by poliovirus is the level of mucosally secreted antibodies, not serum antibody titers. IPV (Inactivated Poliovirus Vaccine) elicits higher serum antibody titers than OPV (Oral Poliovirus Vaccine) but induces lower rates of protection, as measured by virus shedding in stools after challenge with OPV1 [19]. Protection from poliovirus disease (paralysis) is, in turn, achieved by serum antibodies.

Ebola correlates of protection are difficult to identify in humans since they can only be analysed during outbreaks, when the planning and performance of clinical studies is constrained by the emergency situation. In survivors of EVD, both humoral and cellular immunity are detected, however, their relative contribution to protection in humans is unknown [20]. A primary antibody response (IgM) can be detected in the blood of infected persons 2–9 days after infection, whereas IgG antibodies appear approximately 17–25 days after infection. This IgG response coincides with the recovery phase.

Analyses of factors correlating to clinical outcomes of EVD have been performed in cohorts of survivors from the 2014 West Africa epidemic. In EVD survivors, lower levels of inflammation correlating with virus clearance and robust Ebola-virus-specific T cell responses were observed compared to fatal cases. In contrast, fatal cases of EVD were associated with a higher percentage of $CD4^+$ and $CD8^+$ T cells expressing the inhibitory molecules CTLA-4 and PD-1, which correlated with elevated inflammatory markers and high virus load [21]. Transcriptomic analyses of blood RNA from EBOV-infected patients revealed upregulation of interferons and acute phase signaling pathways in groups with fatal disease compared to survivors; moreover, circulating monocytes were significantly lower in fatal cases, while NK cells accumulated in survivors [22]. Of note, CoP may differ between infection and immunization-induced immunity, and although antibodies are believed to be essential for Ebola vaccine-mediated protection (see below: 3.1.2 and 3.1.3), immune correlates of protection against disease have not yet been identified.

Animal model studies are thus of critical importance to help identifying correlates of protection against EVD. Protection from disease and death has been assessed using various animal models (mice, guinea pigs and NHPs) following vaccination with mostly VSV- or adenovirus-vectored vaccines. Both humoral and cellular immunity were demonstrated to be involved in protective immunity in NHP. The relevance of EBOV GP-specific IgG after vaccination with rVSVΔG-ZEBOV-GP was demonstrated in guinea pigs and NHP [23]. Using knock-out mice, protection was demonstrated to be mediated by B cells and $CD4^+$ T cells [23]. In a recent study, anti-GP antibody level and functionality at acidic pH have been shown to correlate with survival of NHPs vaccinated with VLPs expressing EBOV GP, VP40 and NP; this correlation was not observed for anti-VP40 antibodies [24]. Vaccination with recombinant serotype 5 adenovirus encoding Ebolavirus GP also generated anti-GP specific antibodies, which however did not mediate protection alone since the transfer of EBOV GP-specific IgG from Ad5-EBOV vaccinated NHP to naïve animals did not protect them against death after EBOV challenge [25]. In turn, when $CD8^+$ T cells were depleted, 4 out of 5 vaccinated animals succumbed after challenge, suggesting a major role for these cells [25]. These data suggest that different vaccines can mediate protection through different mechanisms, likely with diverse correlates of protection. Importantly, to date it is still difficult to define how well animal models reproduce the characteristics of human EVD, and thus to extrapolate putative vaccine-induced CoPs from animals to humans.

3. Vaccine candidates against Ebola

The 2014 EVD outbreak promoted unprecedented efforts for the development of Ebola vaccines and several vaccine candidates were advanced in clinical studies. There are currently eight vaccines in clinical trials, all targeting the Ebola virus GP [26]. The most advanced vaccine candidate is the rVSVΔG-ZEBOV-GP vaccine, based on the replication-competent VSV expressing only the surface glycoprotein of Zaire Ebolavirus [8,9]. It was developed by the Public Health Agency of Canada (BPSC1001) and licensed to NewLink Genetics and subsequently to Merck Sharp & Dohme. The rVSVΔG-ZEBOV-GP vaccine conferred 100% protection in NHPs and has shown to be reactogenic but safe and immunogenic in human studies [27–31]. It was shown to be protective (at least in the short term) in the ring-vaccination trial performed in Guinea in 2015 [30]. Having been granted Breakthrough Therapy Designation by the FDA and PRIME status from the European Medicines Agency (EMA) [Merck Press Release, 25 Jul 2016], it is now awaiting licensing.

Another advanced vaccine candidate is the ChAd3-EBO vaccine, based on the non-replicating chimpanzee adenovirus type 3 (cAd3) vaccine vector, which encodes for glycoprotein antigens from both the Zaire and Sudan species of *Ebolavirus,* responsible for most EVD outbreaks. This vaccine showed 100% efficacy in NHPs early after immunization [32]. However, this protection waned over a few months, requiring boosting to reactivate protective immunity. The induction of

T cell responses was identified as critical for protection. The recent results of the Phase I clinical studies with ChAd3-EBO-Z indicate that a high vaccine dose (2×10^{11} pfu) is required to trigger antibody responses which appear relatively similar to those elicited by 2×10^{7} pfu of rVSVΔG-ZEBOV-GP [33,34]. A Phase II placebo-controlled trial of both ChAd3-EBO-Z and rVSVΔG-ZEBOV-GP vaccines was conducted in Liberia (NCT02344407) confirming that both vaccines were safe and immunogenic, eliciting antibody responses maintained one year after vaccination in 79.5% of rVSVΔG-ZEBOV-GP and 63.5% of ChAd3-EBO-Z vaccinated subjects [35].

The third candidate, a recombinant adenovirus type-5 vector-based vaccine expressing the glycoprotein of Ebola Zaire Makona variant, which was protective against challenge in NHPs at a dose of 4×10^{10} pfu, also showed good safety and immunogenicity in healthy adults in Sierra Leone [36].

Prime-boost strategies in which priming with ChAd3-EBO-Z was followed by heterologous boosting were evaluated in Europe (NCT02240875, NCT02416453), US (NCT02231866) and Africa (NCT02267109). These studies showed that 1×10^{11} pfu of ChAd3-EBO-Z elicited strong anti-glycoprotein antibody responses in all participants and that boosting with 2×10^{8} pfu of MVA-BN-Filo© increased the titer and duration of antibody responses [37].

Another heterologous prime-boost regimen based on priming with 5×10^{10}pfu of the recombinant Ad26-vectored vaccine which expresses Mayinga EBOV GP (Ad26-ZEBOV) followed by a booster dose (1×10^{8} $TCID_{50}$) of the recombinant vaccinia Ankara–vectored vaccine expressing 3 filoviral glycoproteins (MVA-BN-Filo©, Bavarian Nordic [32]) is under investigation. Studies in NHP demonstrated that a single dose of Ad26-ZEBOV generates only partial protection against challenge [38], necessitating a prime-boost combination. Phase 1 clinical trials to evaluate the safety, tolerability and immunogenicity of heterologous prime-boost regimens using MVA-BN®-Filo and Ad26-ZEBOV administered to healthy adults in different sequences and schedules have been conducted in Europe (NCT02313077), Africa (NCT02376400, NCT02376426) and the US (NCT02325050). They indicated that boosting with MVA- BN®-Filo increased virus-specific antibodies and glycoprotein-specific $CD8^{+}$ T cells [33].

3.1. The rVSVΔG-ZEBOV-GP vaccine candidate

The main focus of this review is the rVSVΔG-ZEBOV-GP vaccine as it is the most advanced vaccine candidate, the only one with demonstrated efficacy in a vaccination trial in 2015, and the one used again for ring vaccination in the current (2018) DRC outbreak. The rVSVΔG-ZEBOV-GP vaccine is nearing the completion of its development phase and is transitioning to a commercial product. The general characteristics of rVSV-based vectors have been reviewed in [8]. Further studies need to be conducted on the safety and immunogenicity of rVSVΔG-ZEBOV-GP in vulnerable populations, such as young children or pregnant women, as well as on the duration of protection and identification of CoPs. Although each vaccine / immunization regimen may differ in its CoPs, the information generated for the rVSVΔG-ZEBOV-GP vaccine is anticipated to help inform the development of other vaccine candidates.

3.1.1. Pre-clinical studies with rVSVΔG-ZEBOV-GP

There is a significant body of pre-clinical safety and efficacy NHP data for the rVSVΔG-ZEBOV-GP vaccine candidate. Protection against EVD by rVSVΔG-ZEBOV-GP was first reported in NHP [39,40] following a single intramuscular (IM) injection of 1×10^{7} pfu. Vaccine vector shedding was not detectable and none of the challenged animals developed overt fever or detectable adverse events [39,41]. Complete or partial protection was even achieved with a single injection of 5×10^{7} pfu administered 7 or 3 days before challenge, respectively, demonstrating that rVSVΔG-ZEBOV-GP may rapidly confer protection and thus further supporting its use for rapid responses during outbreaks [42]. Safety in immunocompromised hosts, an important consideration given the potential presence of HIV infected individuals in the target vaccine populations, was evaluated in a few rhesus macaques infected with simian-human immunodeficiency virus (SHIV): none of the infected animals showed evidence of illness following rVSVΔG-ZEBOV-GP immunization with 1×10^{7} pfu and 4 out of 6 were protected from EBOV challenge [40]. rVSVΔG-ZEBOV-GP also showed an efficacy of 33–67% when injected 24 h post-infection of Rhesus macaques with Ebola virus Makona [43]. Its potential for protection against newly emerging, phylogenetically related strains was assessed by immunizing macaques prior to challenge with Bundibugyo Ebolavirus (BEBOV), a newly emerged EBOV species [44]. A single vaccination with 2×10^{7} pfu of rVSVΔG-ZEBOV-GP provided significant cross-protection (75% survival), suggesting that monovalent VSV-based vaccines may be also useful against newly emerging species [45], although whether this applies to humans remains undefined.

3.1.2. Clinical studies with rVSVΔG-ZEBOV-GP

In August 2014, after the EVD outbreak hit 6 West African countries, the Public Health Agency of Canada donated 800 vials of the rVSVΔG-ZEBOV-GP candidate vaccine to the WHO, who created an African and European VSV-Ebola consortium (VEBCON) to initiate and accelerate phase I trials. VEBCON dose-escalation clinical studies started in Germany (NCT02283099), Kenya (NCT02296983) and Gabon (PACTR2014000089322) while a phase 1/2 randomised controlled trial (RCT, NCT02287480) was initiated in Switzerland. Parallel dose-escalation trials were performed in the US, and field trials subsequently took place in West Africa. To date, the rVSVΔG-ZEBOV-GP vaccine has been tested in eight Phase I and four Phase II/III clinical trials enrolling approximately 17,000 subjects, showing high immunogenicity, safety and at least short-term protective efficacy [10,11,46–50].

Results from the Phase I trials indicated that a single high dose of rVSVΔG-ZEBOV-GP vaccine was immunogenic, safe but also reactogenic [27,28]. At high doses, ranging between 3×10^{6} and 5×10^{7} pfu, mild-to-moderate early onset reactogenicity (fever, myalgia, chills, fatigue, headaches, etc.) affected most subjects. The symptoms were transient, and associated with viremia and haematological changes indicative of vaccine replication [27,28]. However, oligoarthritis was reported in the second week after injection in 11/51 Geneva high-dose vaccinees, occasionally accompanied by vesicular dermatitis. Viral dissemination and replication in peripheral tissues was confirmed by the identification of rVSVΔG-ZEBOV-GP in synovial fluid and skin vesicles [46]. Reduction of the vaccine dose to 3×10^{5} pfu markedly reduced acute reactogenicity, but did not prevent arthritis, dermatitis or cutaneous vasculitis in 12/51 Geneva vaccinees and decreased the magnitude of antibody responses [29]. Initially not observed elsewhere, joint symptoms similarly occurred in 5% of vaccinees in a dose-finding Phase Ib study performed by NewLink in the US [10,11,46,48,49,51]. Arthritis lasted between a few days and several months, but eventually resolved without major sequelae. Vaccine-associated severe adverse events were not reported in any of the rVSVΔG-ZEBOV-GP phase I trials, and rVSVΔG-ZEBOV-GP was advanced in further phase II/III trials by the WHO (Guinea), the US Center for Disease Control (Sierra Leone), the US National Institute of Health (Liberia) along with the National Health Authorities of these countries. The efficacy of a single intramuscular dose of rVSVΔG-ZEBOV-GP (2×10^{7}pfu) was demonstrated in an open-label, cluster-randomised ring vaccination trial (PACTR201503001057193) conducted in the last part of the 2014 outbreak in Conakry, Guinea, and Sierra Leone [10,29]. The study was conducted on a total of 11,841 subjects, assigned to 117 clusters (rings), vaccinated either immediately or 21 days after a known contact to an EVD case. No cases of EVD occurred among vaccinated individuals from day 10 after vaccination showing a 100% vaccine efficacy [11]. About 54% of the vaccinees, across all age groups, reported mostly mild adverse events (headache, fatigue, muscle pain) in the 14 days after vaccination. Another ring vaccination with rVSVΔG-ZEBOV-GP

included 1510 subjects in Guinea and confirmed that a ring vaccination strategy can be rapidly implemented in response to EVD outbreaks in rural settings [52]. The Merck-sponsored phase III efficacy clinical trials (NCT02503202) conducted in about 1200 healthy adults in West Africa confirmed the safety of rVSVΔG-ZEBOV-GP, with up to 80% of vaccinees reporting mild to moderate adverse events (injection-site pain, myalgia, headache, fatigue, fever, and chills). The study also confirmed vaccine-associated joint inflammation, with 4.9% of subjects reporting arthritis and 17.9% reporting arthralgia [31].

In the ongoing 2018 DRC EVD outbreak, the non-yet licensed rVSVΔG-ZEBOV-GP vaccine is being used under the Expanded Access framework to provide access to medical products that have not yet received regulatory approval [12]. MSF is running an open-label single group trial, planning to include 500 people to provide additional information on vaccine safety and efficacy while WHO started a new ring vaccination strategy.

3.1.3. Human immune response to single-dose rVSVΔG-ZEBOV-GP vaccination

Phase 1 and phase II clinical trials conducted in EU and Africa have shown that EBOV GP–specific IgG antibody responses were detected in almost all participants [35,48,50], with significantly higher titres of EBOV neutralizing antibodies at higher vaccine doses [48]. The importance of a predominantly anti-GP IgM response for EBOV neutralization and strong correlation between in vitro EBOV neutralization and serum GP-binding antibody titres have been shown [53]. Administration of a second vaccine dose did not boost antibody or virus neutralization titers and eliciting limited antibody affinity maturation [53]. Correlation of ZEBOV-specific cTfh (circulating follicular T helper cells) with antibody titers and Tfh17 cells was also reported [54]. The team from Hamburg reported the identification of a complex signature including several components of early innate responses as a correlate of antibody responses to rVSVΔG-ZEBOV-GP (see below, paragraph 4, [55]).

An innate signature correlating with both immunogenicity and reactogenicity of rVSVΔG-ZEBOV-GP in human vaccinees from Europe and Africa has been recently identified. This early signature (day 1) includes 6 monocyte-related chemokines and cytokines, indicating that monocyte recruitment and activation play an essential role in the response to rVSVΔG-ZEBOV-GP, shaping subsequent adaptive responses. Remarkably, this plasma signature is strongly correlated with vaccine viremia, vaccine-induced hematological changes, both the occurrence and magnitude of reactogenicity, and arthritis [56].

The persistence of vaccine-induced ZEBOV glycoprotein (IgG) antibodies was assessed in an observational cohort study of the VEBCON African and European phase 1 rVSVΔG-ZEBOV-GP trials (NCT02287480, NCT02933931; NCT02296983 PACTR201411000919191) involving subjects vaccinated once in 2014-15 with 3×10^5 (low dose) or $1–5 \times 10^7$ (high dose) pfu of rVSVΔG-ZEBOV-GP. This study showed that antibody responses to a single-dose of rVSVΔG-ZEBOV-GP were sustained for at least 2 years across dose ranges and settings. This is of critical importance for the development of an Ebola vaccine to be used in countries where booster vaccinations would be difficult to conduct [57].

4. Systems biology analysis

New technologies such as RNA sequencing allow a deep characterization of gene expression patterns in samples from vaccine trials or from EBOV-infected subjects and animals. Gene expression data can be integrated with immunologic parameters such as humoral and cellular responses in order to provide a bigger picture of the immune response, to understand mechanisms of vaccine immune response and to identify new, measurable CoPs of vaccine-mediated protection. Such systems vaccinology approaches have been successfully applied to a number of vaccines [58–60] and allowed the identification of predictive gene signatures of both antibody response and antigen-specific $CD8^+$ T cell response, as early as 7 days after yellow fever vaccination [61]. A recent study on malaria vaccines, using 2 different prime-boost immunization strategies followed by controlled malaria challenge on human volunteers, identified molecular signatures of protection from infection: in challenged but protected individuals, molecular signatures related to NK cells were negatively correlated with protection [60].

Analyses of the transcriptomic response to rVSVΔG-ZEBOV-GP were first conducted in animal studies investigating protection after viral challenge. Immunized and subsequently challenged animals showed a limited modulation of gene expression compared to unprotected animals. The response was centered on innate immunity pathways (interferon signaling, antiviral response) and on T cell receptor signaling, suggesting a participation of both adaptive and innate immunity in the recall response elicited by ZEBOV challenge [62]. Another transcriptomic study suggested a role for $CD8^+$ T cells as mediators of protection induced by rVSVΔG-ZEBOV-GP in NHPs, although this needs further validation [63].

Systems vaccinology analysis of human responses to rVSVΔG-ZEBOV-GP in 20 vaccinees from Germany revealed a strong correlation between the serum levels of IP-10 at day 3 after vaccination and EBOV-GP-specific antibodies measured until day 180 after vaccination. The expression levels of 15 genes on different days after vaccination were predictive of the later antibody response, among them the *TIFA* gene which is related to the IP-10 pathway [55]. Early activation of innate immune cells was also reported: activation of NK cells was positively correlated with antibody titers, while activation of monocytes and DC was negatively correlated [55].

Novel data from human transcriptomic studies on rVSVΔG-ZEBOV-GP vaccination suggest that ZEBOV-GP-specific antibody titers at 1 year after vaccination correlate with the levels of expression of a transcriptional regulator which is specifically expressed in human effector lymphocytes and in tissue resident T-cells (Santoro et al. *manuscripts in preparation*). Preliminary analysis of whole-blood transcriptomic data 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*).

Further, Gas Chromatography Mass Spectrometry combined with a systems biology approach was used to pinpoint metabolic changes induced by rVSVΔG-ZEBOV-GP in the plasma of 115 healthy volunteers enrolled in phase 1/2, placebo-controlled, double blind safety and immunogenicity trials of rVSVΔG-ZEBOV-GP in Geneva (NCT02287480). Results showed that plasma levels of a group of metabolites significantly changed following vaccination; some of these correlated with ZEBOV GP specific IgG antibody responses detected after vaccination (Olafsdottir et al, *manuscript in preparation*). These studies were carried out in the context of two new Ebola vaccine projects, which will be discussed in Section 5. Integrative analyisis of clinical, immunological, transcriptomic and metabolomic data obtained from animal models, vaccine human clinical trials and from EVD survivals will be essential to identify and validate molecular signatures correlated with protection from infection (Fig. 1).

5. The VSV-EBOVAC and VSV-EBOPLUS efforts

In response to the 2014 EVD outbreak in West Africa, the Innovative Medicines Initiative 2 Joint Undertaking (IMI2 JU, http://www.imi.europa.eu/), launched the "Ebola and other filoviral haemorrhagic fevers" (Ebola+) programme. In this context, the VSV-EBOVAC (www.vsv-ebovac.eu) and VSV-EBOPLUS (http://www.vsv-eboplus.eu) projects were launched to characterize the immune and molecular signatures induced in humans by the rVSVΔG-ZEBOV-GP vaccine [64]. VSV-EBOVAC has conducted transcriptomic and metabolomic analysis on more than 400 blood samples obtained at different time points following immunization with rVSVΔG-ZEBOV-GP in Switzerland, Kenya and Gabon. Samples from the same individuals have also been analyzed in depth to characterize innate and adaptive immune responses. A

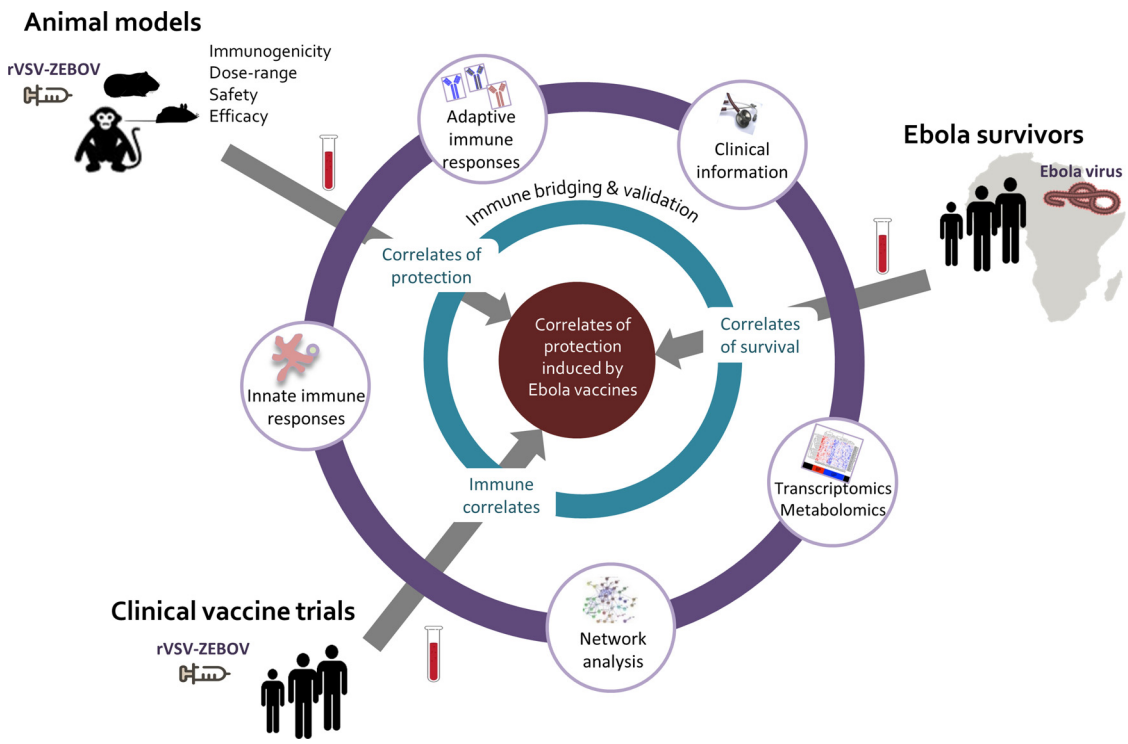

Fig. 1. Integration of data from animal models, vaccine clinical trials and Ebola survivors to identify Ebola vaccine correlates of protection.
Animal models (mice, guinea pigs, NHPs) can be used to assess vaccine correlates of protection after virus challenge. Vaccine clinical trials in humans can assess vaccine immunogenicity and safety as well as efficacy during outbreaks, data from survivors and non-survivors during epidemics can be used to establish markers of survival and to investigate the development of a protective immune response. Different analyses are used to investigate the response to vaccination/infection: collection of clinical data, immunological analysis, assessment of transcriptomic and metabolomic profiles in blood. All these data need to be integrated together using a systems biology approach to identify correlates of protection from infection.

systems biology approach is being used to integrate data obtained from different platforms in order to identify molecular patterns and pathways involved in vaccine response. The VSV-EBOPLUS project conducts immune monitoring beyond the demonstrated (short) duration of protection and compares responses and signatures from children (in whom efficacy could not be assessed in the absence of an outbreak) to those identified in adults. These efforts build on the rVSVΔG-ZEBOV-GP phase I trials performed in Geneva, Lambaréné and Kilifi that 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. This in depth integrated analyses of data will allow to fully exploit and share all the information generated from the clinical studies. The ongoing use of the rVSVΔG-ZEBOV-GP vaccine during the current 2018 outbreak in DRC could offer further opportunities to elucidate its correlates of protection, although it appears that biological samples are unfortunately again not harvested from the vaccinees. Results obtained from an integrated omics approach, combined with clinical and immunological read outs, could lead to the discovery of new molecular biomarkers and immune correlates of rVSVΔG-ZEBOV-GP vaccine safety and immunogenicity.

The VSV-EBOVAC and VSV-EBOPLUS projects are implemented in close synergy and complementarity with the 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) [65]. These joint efforts are in line with the Roadmap on Vaccines in Europe (IPROVE) [66] and are expected to provide novel data to support the development of rVSVΔG-ZEBOV-GP as a safe and efficacious Ebola vaccine and to advance the knowledge of Ebola vaccine CoP.

6. Conclusions

Despite significant progress in the characterization of the response to vaccination, CoP against Ebolavirus infection have not been established in humans. This holds true even for rVSVΔG-ZEBOV-GP, the most advanced Ebola Vaccine candidate and the only one with demonstrated efficacy in humans. Innate and adaptive immunity both contribute to rVSVΔG-ZEBOV-GP responses, but how these combine to protect early after exposure and whether the same mechanisms will contribute to long-term protection is undefined. Unfortunately, integrating research components to advance the understanding of vaccine-induced efficacy and characterise its CoP proves challenging in emergency outbreaks settings, and major efforts would be required to ensure that samples and data are collected. Integration of data from pre-clinical and clinical vaccine studies together with data from disease survivors will thus be essential to identify Ebola vaccine correlates of protection. The information generated for rVSVΔG-ZEBOV-GP may help identify the CoP of the other Ebola vaccine candidates – although these may also differ.

Declarations of interest

None.

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