Heterologous prime-boost immunisation in Ebola vaccine development, testing and licensure

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Several of the leading Ebola vaccine candidates are replication deficient recombinant viruses. These include chimpanzee adenovirus 3 (ChAd3), human adenovirus 26 (Ad26) and modified vaccinia virus Ankara (MVA), all bearing the gene for ebola glycoprotein. In addition to these vaccines, there is a replication competent viral vector, vesicular stomatitis virus (VSV) vaccine.

For the first three vaccines (ChAd3, Ad26, MVA), there is strong pre-clinical data showing that heterologous prime-boost approaches can induce superior antibody and T cell responses than repeated dosing with the same vaccine.

Single dose vaccines are highly preferable for both price and operational/logistic considerations, particularly when considering low income country settings. Therefore, it is understood that compelling data will be necessary from clinical trials that heterologous prime-boost combinations are required and are of added benefit to single dose vaccination regimens for initial efficacy and/or the duration of protection that they provide before they are considered for use on a large scale.

Against this background, the World Health Organization convened a meeting on 21 November 2014 of vaccine scientists, clinical trialists, vaccine developers and regulators to consider the key questions to be addressed in Phase 1 clinical trials of Ebola vaccines in order to generate data that can be used for decision-making for more advanced clinical development and testing.

Advanced planning is underway for Phase 3 trials of Ebola vaccines, and given the public health imperative and ongoing humanitarian crises in the 3 most heavily affected countries in West Africa, timelines for Phase 3 trials, potential licensure and use are being accelerated by an international partnership coordinated by WHO. Therefore, the discussions held at the meeting were intended to reflect the need to generate Phase 1 clinical data in short timeframes without delaying the ongoing planning and initiation of Phase 3 trials. Phase 1 data that are available by January might influence the design of Phase 3 trials due to start in January to February of 2015.

What is heterologous prime-boost?

The terms prime and boost are often used in vaccinology to describe the induction of an enhanced immune response in subjects given a ‘booster’ dose of the same vaccine used to induce a primary immune response. However, ‘heterologous prime-boost’ has a specific
meaning, describing the use of two different vaccines in sequence to induce improved immunogenicity and efficacy compared to two doses of the same vaccine. The two vaccines in a heterologous prime-boost combination present the same or overlapping antigenic components to the immune system but use different delivery systems or vectors. This avoids the potential inhibition of the response to a second dose of the same vector, due to neutralizing antibodies against the vector induced by the first dose.

How much do we know about heterologous prime-boost in humans?

It was shown in multiple animal models and clinical trials\textsuperscript{1-3} during the 1990s that the heterologous prime-boost approach can induce superior immune responses, especially T cell responses, compared to repeated dosing with the same vaccine.

This approach has been explored most extensively in malaria, where a field efficacy study is ongoing in infants in Burkina Faso of an adenovirus prime and poxvirus boost regimen; the first field efficacy study of a heterologous prime-boost malaria vaccine regimen dates back to 2002\textsuperscript{4}. For HIV, the leading approach under test is based on heterologous prime-boost of protein and poxvirus, the RV144 concept, for which efficacy was demonstrated in 2009\textsuperscript{5}. Non-human primate data has also supported the adenovirus prime-poxvirus boost approach for vaccination against HIV\textsuperscript{6}. In tuberculosis, a recent field efficacy study was conducted in South Africa using BCG and a poxvirus boost\textsuperscript{7}, albeit without evidence of efficacy. In addition, there are some clinical data for hepatitis C\textsuperscript{8} and other indications.

At the meeting, a review of the clinical experience with the heterologous prime boost approach indicated that over 100 clinical trials have now occurred with heterologous prime-boost vaccine combinations over the last 20 years. Some of these studies have included several hundred subjects, including adults, children and infants in sub-Saharan Africa. The largest single heterologous prime-boost trial was that of RV144 in Thailand, with approximately 16,000 study subjects.

Foreseeing that the use of prime boost vaccination regimens would become an important topic for evaluation and potential licensure, WHO organized two consultations on heterologous prime-boost and recombinant viral vectors during 2012 and 2013. The first was jointly convened with NIAID. These highlighted the following points:

- It is possible that heterologous prime-boost regimens will be required for effective vaccination against several infections in order to afford efficacy at some point in the future, and therefore it is timely for regulatory authorities to consider how they will license “Vaccine 1 followed by Vaccine 2” for the same indication.
- While single dose vaccines are ideal, and repeated doses of the same vaccine are preferable to heterologous prime-boost from an implementation perspective, the feasibility of implementation for heterologous prime-boost would not provide an insurmountable barrier to licensure and use if it was shown that there are sufficient
public health benefits to justify the added complexity of implementation. However, a careful analysis of the pros and cons would need to be made before making recommendations for use of heterologous prime-boost approaches.

There has been no specific safety issues related to use of the heterologous prime-boost approach. However, it was considered appropriate by those at WHO consultations that some data should be obtained from small clinical studies on reverse order immunization (for lack of harm) and on repeated dosing with the same vaccine to demonstrate superior immunogenicity of heterologous prime-boost vs repeated homologous dosing as well as to establish the safety of non-recommended regimens that might be given inadvertently.

*What is the aim of heterologous prime-boost vaccination in the prevention of Ebola infection?*

Reasons for considering the prime-boost approach for the prevention of Ebola infection include -

1) Initial efficacy may be inadequate for public health use following a single dose or two doses of the same vaccine.
2) The duration of protection following a single dose may be short-lived with some vaccines (for example protection as short as 3-6 months is possible) and populations at risk may require protection for longer periods.

Heterologous prime-boost regimens may overcome both of these potential concerns. A recent publication reported increased antibody and T cell immunogenicity, and superior efficacy and duration of protection against Ebola challenge with ChAd3-MVA compared to ChAd3 alone in non-human primates.9

*What is the priority heterologous prime-boost combination for protection against Ebola, what are the optimum doses of each vaccine and what is the optimum gap between doses?*

In the case of the VSV Ebola vaccine, which is based on a replicating virus, there are no pre-clinical data which suggest the need for heterologous prime-boost vaccination and the manufacturer of the most advanced VSV candidate Ebola vaccine confirmed at the meeting that their company does not see value in exploring heterologous prime-boost combinations at the present time.

There was consensus among those present at the meeting that the priority combinations for testing in Phase 1 clinical trials are the ChAd3-MVA and Ad26-MVA combinations, and that these heterologous prime-boost combinations may add substantial benefits above single dose vaccination. Both combinations have demonstrated 100% short-term protection against Ebola infection in macaques and ChAd3-MVA has conferred durable protection up to 10 months after vaccination, whereas protection provided by the ChAd3 vaccine alone was waning at 10 months post-vaccination in the macaque model. There is a large body of pre-
clinical and clinical data indicating that the adenovirus prime-MVA boost regimen is an excellent strategy for maximising dual antibody and T cell immunogenicity, with some efficacy data for malaria vaccines, and non-human primate efficacy for HIV vaccines, supporting use of this approach.

It was agreed that the ChAd3-Ad26 and Ad26-ChAd3 combinations, for which there is some supporting pre-clinical data, also warranted evaluation in man although with less priority than that for the ChAd3-MVA and Ad26-MVA combinations. It was agreed that it is important to keep options open by generating Phase 1 data on multiple combinations, given the uncertainties over the ability of manufacturers to scale up production of some vaccines. Ad26 is manufactured using a cell line and so may be more amenable to large scale production than MVA that uses a chick embryo fibroblast-based (egg) manufacturing process. However, it was stated at the meeting that a different manufacturer is commencing MVA EBO Z manufacture using a cell line, aiming for at least 10,000 doses to be available by late February 2015.

The optimum dose of MVA for boosting is known to be in the $10^8$ pfu (plaque forming unit) range. Doses for ChAd3 and Ad26 could be of the order of $2.5 \text{ to } 5 \times 10^{10}$ virus particles (vp) for the prime in Phase 1 trials, although it was considered that a $1 \times 10^{11}$ Vp priming dose may also warrant evaluation.

It was agreed that intervals between doses ranging from 2 weeks to 12 weeks merit evaluation. Clinical data suggest that MVA can be used to successfully reboost immunity at 6-12 months after primary immunisation.\(^\text{10}\) This could be a way to extend protection further, but would need to be evaluated in clinical trials as this may be an antigen-specific effect.

**Regulatory considerations**

The regulatory representatives at the meeting from US FDA and EMA stated that there are no specific barriers to licensure or marketing authorisation of heterologous prime-boost vaccine combinations. Co-development of two different vaccine candidates considered for heterologous prime-boost will generally require less information about the clinical safety and effectiveness of the individual vaccine candidates than would be required if the individual vaccine candidates were developed alone. Therefore, a full data package supporting the safety and effectiveness of the individual vaccine candidates would not be necessary if licensure of the individual vaccine candidates is not pursued. Instead, the clinical development should focus on demonstrating the safety and efficacy of the combination to support a marketing authorization. The indication sought will determine the requirements to some extent. Thus, the recommendation was that manufacturers should proceed with heterologous prime-boost regimens when clinical data show that they are necessary and of added benefit to vaccination with one vaccine alone, in close discussion with the relevant regulatory authorities.
Implementation of a prime-boost regimen

The meeting was designed specifically to address scientific questions in relation to Phase 1 clinical trials and there were consequently only limited discussions about implementation of prime-boost regimens. However, the team at WHO that is responsible for coordinating cholera vaccine mass campaigns presented their experience with two doses of this oral vaccine which are given 14 days apart. Because of the perception of cholera as a very serious disease, good coverage with 2 doses has been achieved, including experience during an epidemic in Guinea, where approximately 15% drop-out rates were seen from first to second dose. However, it was noted that there is little public health experience with 2-dose parenteral mass campaigns, let alone heterologous prime-boost combinations.

It is clear that further exploration and preparatory on-the-ground work on the programmatic feasibility of administering “Ebola vaccine 1” followed by “Ebola vaccine 2” is needed, although it would be premature to prioritise such activities, given all the other competing priorities at this time, until clinical data indicate whether or not heterologous prime-boost is required.

Assay standardization and comparability of immunogenicity readouts between centres

It was agreed that adenovirus-based programmes should assess both antibody and T cell immunogenicity. Non-human primate data from Ebola vaccine studies presented at the meeting suggested that IgG binding antibody titres can be considered a non-mechanistic correlate of protection in NHP immunized with the adenovirus 5–based vaccine. Neutralising antibody concentrations did not correlate well with protection. In this experimental system, protective immunity could not be transferred with serum, but CD8 T cell depletion completely removed efficacy from subsequent challenge. Nevertheless, IgG titres are strongly associated with protection, with all animals that achieved greater than approximately 3500 IgG ELISA units protected. Thus, it will be imperative that IgG ELISA data, cross-referenced adequately to the in-house NIH assay used in the macaque trials and T cell assays such as ELISpot and intracellular cytokine staining, are generated in Phase 1 trials. Evidence was presented during the meeting that the qualitative characteristics of T cells, namely the number and specificities of the cytokines that they produce may also be related to protective efficacy. It was noted at the meeting that the amount of ebola virus administered to macaques in challenge experiments is probably 10-100 fold higher than the dose of Ebola virus received in natural exposure of humans. This could affect how preclinical challenge efficacy translates to clinical efficacy results both in terms of vaccine regimens that can protect, and the correlates of protection for human naturally acquired Ebola virus infection.

As in all vaccine development, standardization will be very important, and the multiple ongoing activities in this field were outlined at the meeting. These include activities by at least two laboratories that are conducting immunoassays on sera from multiple Phase 1
trials. These are a GLP-accredited laboratory at the University of Marburg, facilitated by WHO, which uses as antigen Guinea 2014 Ebola virus isolate in a validated IgG ELISA assay, and secondly a laboratory at the NIH Vaccine Research Center which uses a secreted trimeric Ebola glycoprotein as antigen. The latter assay has the benefit of being more closely related to the assay used in non-human primates for preclinical challenge experiments with ChAd3 and ChAd3-MVA.

NIBSC is starting on a process to develop standard reagents including an IgG calibration reagent for use in vaccine, blood product and convalescent serum clinical trials, as well as development of a reference panel of multiple reagents.

**Conclusion**

It remains unknown what the initial efficacy and duration of protection of the Ebola vaccines currently under development will be. Certain vaccines may not require heterologous prime-boost combinations, and single dose vaccination regimens will likely be preferred providing that quality, safety, efficacy, and supply are all favourable. A priority for both ChAd3 and Ad26 will be to evaluate in Phase 1 studies whether MVA boosting at the $10^8$ pfu dose level and focusing on 4-8 week intervals is superior for antibody and T cell immunogenicity than the priming dose alone.

It was confirmed that ChAd3-MVA Phase 1 data in Ebola would be generated soon, with MVA boosting during late November and December 2014.

Ad26-MVA Phase 1 data would be generated with the prime in late December, and the boost in January 2015.
References


