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Consensus considerations on the assessment of the risk of disease enhancement with **COVID-19** vaccines:

Outcome of a Coalition for Epidemic Preparedness Innovations (CEPI)/Brighton Collaboration (BC) scientific working meeting, March 12-13, 2020

Background

The SARS-CoV-2 pandemic presents an unprecedented challenge to global health with enormous health system, social and economic disruption and large numbers of deaths already experienced in many countries. Epidemiologic features confirm that ongoing spread to less affected areas is now a certainty.

Several academic and commercial vaccine developers are moving quickly, with many different vaccine strategies including RNA, DNA, protein and viral vectored vaccines, and are planning to enter Phase I trials and then upscale to measure safety and efficacy with the intention of vaccine deployment. While some programs will not reach human studies in 2020, others will be in Phase I in days.

The enhanced pathology seen in some SARS or MERS-CoV vaccinated animals when subsequently challenged with live virus was primarily seen in the lungs. It was described as a lymphocytic and eosinophilic infiltrate; an observation similar to the enhanced RSV Disease (ERD) described in infants exposed to natural RSV infection following immunization with a poorly efficient formalin-inactivated RSV vaccine in 1967. CEPI and the CEPI-funded Brighton Collaboration Safety Platform for Emergency vACcines (SPEAC) project convened a working meeting consisting of a group of experts (Working Group) in the field of vaccine immunology and coronaviruses (CoVs) to consider some of the evidence from previous animal studies of MERS and SARS CoVs and initial data with the novel SARS-CoV-2. Presentations and discussions were held via a videoconference on March 12 and 13, 2020. This document summarizes the draft considerations including input from a panel of peer reviewers and additional participants who attended the conference.

The considerations in this document should be interpreted as general scientific remarks based on current knowledge to inform a research agenda that could be beneficial for vaccines development. These considerations are not of a regulatory nature and cannot in any sense replace the need for proper regulatory consultations on the requirements for vaccines clinical trials. Vaccine developers are therefore encouraged to seek individual scientific advice from regulatory authorities.

Animal models

Murine models for assessment of vaccine-related disease enhancement

- SARS-CoV-2 has a low affinity for murine ACE2 receptor and murine models will require the use of hACE2 transgenic mice, preferably with a 'knocked-in' approach. Preliminary data indicate the possibility of infecting hACE2 transgenic mice with demonstration of viral replication and mild lung lesions. Mouse adaptation of SARS-CoV-2, as done with SARS-CoV, will likely be required to obtain a virus that causes more severe disease in mice.
- Previous studies from SARS-CoV and MERS-CoV indicated that some vaccines, especially those using whole inactivated virus, could enhance the disease induced in mice challenged with SARS or MERS. The lung lesions were highly inflammatory, with a dominance of eosinophil infiltration and occurred in animals despite presence of a neutralizing antibody response and with a reduced challenge virus replication in the lungs. Such studies have not yet been completed for SARS-CoV-2.
- In mice, this immunopathology was considered a consequence of a dominant Th2 type response to the vaccine antigens. It was not seen after including adjuvants (e.g. CpG) in the vaccine or other vaccine formulations known to drive immune responses towards Th1. The timing of challenge after vaccination may be critical. It would be of major interest to explore the outcome following challenge at later timepoints when antibodies are significantly decaying.
- One should be aware of the potential confounding effect of cell-culture impurities in the vaccine and challenge strain material. It is known that impurities such as fetal calf serum in the preclinical vaccine preparation may induce eosinophil influx in any mouse model if the challenge strain also contains the same impurities.
- In these models, characterization of the immune response to the candidate vaccine (e.g., IgG isotypes, Th2 markers) may have some predictive value.
- Other small animal models which can be infected by SARS-CoV-2 can be considered (e.g. ferret, hamster).

Non-human primate models for assessment of vaccine-related disease enhancement

- Non-human primates (NHP) are of primary interest in view of their ACE2 homology with hACE2. Preliminary studies indicate the possibility of inducing some COVID-19 lung pathological features after infection, without clinical signs, in Rhesus macaques. African Green monkeys may be more susceptible to COVID-19, but the model suffers from some limitations (e.g. access, genetic polymorphism).
- Previous studies with SARS candidate vaccines have suggested a risk of enhanced pathology in NHPs after viral challenge. Eosinophilic infiltrates were not prominent. The mechanism is still incompletely defined but there is evidence for a role of non-neutralizing antibodies. Non- or incompletely-neutralizing antibodies may contribute to:
    - the formation of pathogenic immune complexes and
    - Fc-mediated viral capture by monocytes/macrophages that may favor excessive T-cell activation and inflammation.
- Enhanced disease could be reproduced by passive transfer of IgG from immunized NHPs.

General considerations on animal models

- Although existing animal models of COVID-19 imperfectly reproduce the human disease, they appear useful for assessing the risk of disease enhancement. Vaccine responses are closer to human responses in NHPs than in mice. Therefore, it is likely that data obtained from NHP studies are more significant. However, there is an urgent need to standardize the NHP model (read-out of disease enhancement, timing of challenge, age) and to include appropriate controls. It is important to control for potential co-infection, including with other coronaviruses, in all non-SPF models.
- Potential markers of safety in these animal models could include:
    - the relative levels of neutralizing vs non-neutralizing antibodies,
    - antibody affinity,
    - T-cell response profile,
    - characterization of lung histopathology.
- Passive transfer in NHPs of human antibodies generated during Phase 1 trials, followed by viral challenge should be considered to assess the risk of disease enhancement.
- Challenge of immunized animals with a closely related heterologous coronavirus strain may assess the risk of enhancement during future outbreaks.
- In case of disease enhancement, in-depth studies in animal models may give some indications on the mechanism of immunopathology. They can inform human trial designers on the critical immunological risk markers to be monitored in Phase 1 trials.
- Based on previous experience with SARS and other viral diseases, the risk of disease enhancement for COVID-19 vaccines (particularly those including whole virions or N protein) could be evaluated in an established NHP model before advanced clinical development.

Potential implications of immunopathology for vaccine design:

Presentations by the Experts covered the following additional key issues

- There are some in vitro and limited in vivo data which suggest that non-neutralizing antibodies might lead to an antibody-dependent enhancement of disease in a feline CoV model. Enhanced disease upon challenge post-immunization has been described for a number of SARS and MERS vaccine antigen/adjuvant constructs in animals.
- Recent data imply that neutralizing antibodies could be induced by the receptor-binding domain (RBD), the N-terminal domain (NTD) and stalk region of the spike protein, and that NTD antibodies may be the most cross-neutralizing.
- A stabilized pre-fusion form of the spike protein may provide higher neutralizing antibodies.
- Antibodies induced against a wider range of epitopes on the spike protein may reduce the risk of viral escape through subsequent mutations.
- The use of adjuvants or vaccine platforms that are likely to induce a Th1 response should be considered in order to enhance neutralizing antibody levels and reduce the risk of enhanced disease.

- Low dose vaccines in animal models for respiratory viruses have generally induced lower immunity rather than inducing immunopathology.
- Animal models appear to mirror human data in that older animals seem more likely to suffer worse disease although respective pathogenic mechanisms in animals and humans may differ in this regard.

The group of Experts suggested that consideration should be given to the following:

- Caution should be observed when developing vaccines to avoid inducing predominant Th2 responses and low levels of non-neutralizing antibody.
- Vaccines inducing strong neutralizing antibodies, predominant Th1 responses and balanced CD4/CD8 and polyfunctional T cell responses are less likely to induce immunopathology.
- Given what will be the unprecedented demand for an effective vaccine, the use of adjuvants may be critical for sub-unit vaccines in providing increased immunogenicity, breadth of response, dose-sparing, duration of response, potentially cross-protection against new CoV strains, and possibly minimization of the risk of enhanced disease. Preference should be given to Th1-driving adjuvants with an established safety profile in humans.
- An understanding of the role of cross-reacting antibodies from prior coronavirus infections may have on natural disease caused by SARS-CoV-2 or influence the risk of enhanced disease following vaccination would be of importance.
- Data are needed on whether antibody waning could increase the risk of enhanced disease on exposure to virus in the long term.

It was the opinion of the Experts that animal data to support clinical development could address:

- Post-vaccination (neutralizing) antibody responses, and T cell analysis to demonstrate a Th1 response.
- Post-vaccination challenge data from NHPs with careful evaluation for immunopathology in the animals.
- Small animal data may also provide important supporting evidence of safety, and both ferret and mouse models are likely to be available for developers.
- Where possible, immunopathology experiments with a positive control (e.g., formalin inactivated alum-adjuvanted CoV vaccine) and most importantly a negative control will provide best guidance. It was felt that it will be important to establish broadly accepted endpoints and scoring systems to allow comparison of various vaccine candidates. WHO is working on this issue.
- For vaccine constructs likely to induce a Th2 responses, the group felt that animal studies should be considered before entering human Phase 1 trials in more than one animal species where possible. It was noted that the absence of a Th2 response does not eliminate the risk of enhanced disease.
- For vaccine constructs which are already known to induce neutralizing antibody and Th1 responses, it was the consensus of the group that while Phase 1 studies are cautiously proceeding

with careful review of safety data, animal studies run in parallel could provide useful information for the further clinical development

- Suggestive data in animal models should not by default prevent clinical development of vaccine candidates; potential risk should be thoroughly evaluated by developers and their regulators on a vaccine product-specific basis**.**

Regarding Phase 1 clinical trials, it was the opinion of the Experts that

- Since studies about to begin will not prescreen to determine preimmunization serostatus of participants, and this shall be determined retrospectively, appropriate baseline blood specimens should be obtained and stored. Because the virus is spreading rapidly, such specimens will allow assessment of the immune response in both seronegative and seropositive persons as both are likely to be vaccinated.
- Level of neutralizing antibodies and determination of the ratio of binding/neutralizing antibodies will be important to assess the potential risk of enhanced disease. Also, detection of initial priming that includes some CD8 T cells and/or a CD4 Th1 biased response is likely to mitigate the risk of disease enhancement. Determination of memory responses will be useful, particularly if SARS-CoV-2 continues to circulate.
- Consideration should be given to the use of post-vaccination sera from vaccinees which could be used for antibody transfer studies in animals to look for enhanced disease and for evidence of cross-protection against other coronaviruses.
- Monitoring for enhanced disease in immunized participants may require longer follow-up than is usual in Phase 1 trials but need not delay Phase 2 trials.
- Investigators on the call requested frequent updating with both preclinical and evolving clinical data that are being developed by the different academic and industrial developers to help in decision-making about the various vaccine clinical trials. Creation of a central information hub was encouraged for this purpose.
- Participants on the call expressed the need for standardization of protocols, data collection forms, critical assays (including reagents) and biobanking of samples from initial clinical trials to allow future re-assay once standards are agreed to and enable comparison of results across trials

Concluding remarks

- The group of Experts considers that the demonstration of some disease enhancement with any candidate vaccine after viral challenge in animal models should not necessarily represent a no-go signal for deciding whether to progress into early trials in clinical development of a COVID-19 vaccine.
- Continuous monitoring of this risk during clinical trials in an epidemic context will be needed.
- Each observed effect should be discussed by the developers with their regulators who will ultimately define the actual requirements for clinical studies.