Viewpoint

Developing a Low-Cost and Accessible COVID-19 Vaccine for Global Health

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Abstract

There is an urgent need to advance safe and affordable COVID-19 vaccines for low- and middle-income countries of Asia, Africa and Latin America. Such vaccines rely on proven technologies such as recombinant protein-based vaccines to facilitate its transfer for emerging market vaccine manufacturers. Our group is developing a two-pronged approach to advance recombinant protein-based vaccines to prevent COVID-19 caused by SARS CoV2 and other coronavirus infections. One vaccine is based on a yeast-derived (*Pichia pastoris*) recombinant protein comprised of the receptor binding domain (RBD) of the SARS-CoV formulated on alum, and referred to as the SARS-CoV 219-N1 Vaccine. Potentially this vaccine could be used as a heterologous vaccine against COVID-19. A second vaccine specific for COVID-19 is also being advanced using the corresponding RBD of SARS-CoV-2. The first antigen has already undergone cGMP manufacture and is therefore "shovel ready" for advancing into clinical trials, following vialing and completing required GLP toxicology testing. Evidence for its potential efficacy to cross-protect against SARS-CoV-2 includes cross-neutralization and binding studies using polyclonal and monoclonal antibodies. Evidence in support of its safety profile include our internal assessments in a mouse challenge model using a lethal mouse adapted SARS strain, which show that SARS-CoV RBD 291N1 (when adsorbed to Alhydrogel®) does not elicit significant eosinophilia or eosinophilic lung pathology. Equally important, we summarize a robust set of published studies linking eosinophilic immunopathology to virus-vectored vaccines. These include vaccinia and modified vaccinia virus Ankara constructs, rather than recombinant protein-based vaccines. Together these findings suggest that recombinant protein-based vaccines based on the RBD warrant further development to prevent SARS, COVID-19 or other coronaviruses of pandemic potential.

The thing we have to think about now that's different is, how do we produce vaccines specifically for the developing world if this is a truly global epidemic. — Seth Berkley, CEO, Gavi

Introduction: Disease burden in low- and middle-income countries

As of March 2019, COVID-19 caused by the SARS CoV2 coronavirus has infected more than 600,000 people globally (confirmed cases) and almost 30,000 deaths (1). Although the largest number of infected patients live in the United States, China and Italy, there are significant concerns about the risks of disease emergence in low- and middle-income nations. There are now more than 35,000 cases in Iran, and although there are currently fewer than 5,000 cases each in India, Indonesia, Brazil, and Sub-Saharan Africa (1), SARS CoV2 infection is expected to emerge in the Global South (2, 3). In the African region of the World Health Organization (WHO), almost 40 countries are now reporting cases (2), while in nations such as India for example, the feasibility of enforcing social distancing in large and crowded urban centers will be particularly daunting (3), so that ensuring access to a safe and affordable COVID-19 vaccine will become a global priority. Dr. Seth Berkley, the CEO of Gavi, the Vaccine Alliance, has highlighted the importance of prioritizing a COVID-19 vaccine specifically for these countries (4).

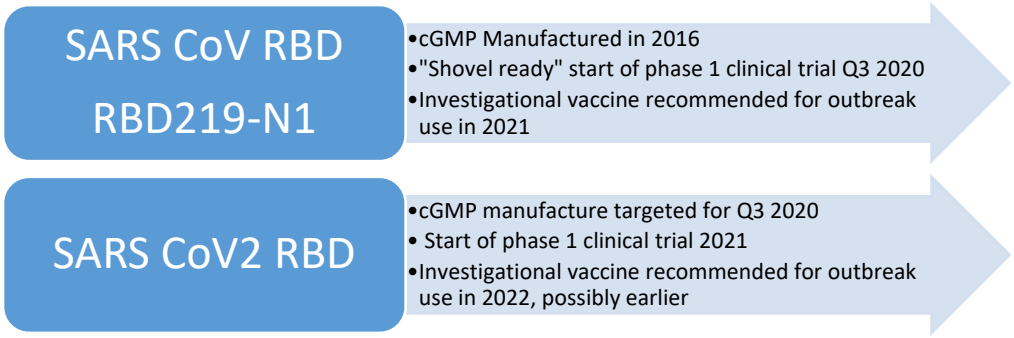
Rationale and approach

At least a dozen COVID-19 candidate vaccines are under development using different technology platforms (5), with an emphasis on speed, maximizing safety and avoiding vaccine-induced immunopathology (6). Many of these will enlist cutting-edge nucleic acid delivery technologies and other innovative approaches. In the meantime, there is urgency to address and rapidly respond to Gavi's charge and pursue safe, low cost, easily administered, and rapidly scalable approaches. For instance, Texas Children's Center for Vaccine Development at Baylor College of Medicine in collaboration with its product development partners have been spearheading a coronavirus vaccine program focusing on recombinant subunit protein vaccines produced in a globally available microbial fermentation platform, and optimized to maximize yield following expression and protein purification (7, 8).

Towards this goal, we are now also developing the SARS-CoV-2 receptor-binding domain (RBD) recombinant protein as a potential vaccine candidate, in parallel with the existing SARS-CoV RBD candidate vaccine, which was previously developed and manufactured under current good manufacturing practices (cGMP) in 2016 (7-9). The bulk drug substance has been stored frozen (-70°C to 80°C) and remains stable through ongoing testing. Furthermore, an independent quality assessment confirmed the suitability of the material through Phase 2 clinical trials.

Both RBD vaccine candidates have potential as vaccine antigens to prevent SARS-CoV-2 infection and/or COVID-19. Overall, our initial approach relies on advancing the already manufactured SARS CoV RBD219-N1 as a heterologous recombinant subunit vaccine to protect against both SARS and COVID-19, and in parallel accelerate the advancement of the SARS-CoV-2 RBD candidate as a homologous COVID-19 vaccine (Fig 1).

Fig 1. Estimated timelines of the SARS-CoV and SARS-CoV-2 RBDs as COVID-19 vaccines (Fig 1)



The SARS-CoV protein known as RBD219-N1 was selected on its ability to elicit high titers of neutralizing antibodies against both SARS-CoV pseudotype virus and live SARS-CoV virus (7, 8), prior to confirmatory testing against SARS-CoV challenge in animal models. It also induced high-level neutralizing antibodies and protective immunity with minimal immunopathology in mice after a homologous virus challenge with SARS-CoV (MA15 strain).

There are several advantages of the RBD219-N1 candidate antigen and vaccine for purposes of global health:

1. ***High yield and low cost***. The antigen is expressed in *Pichia pastoris*, a low-cost expression platform, which can be produced and scaled at high yields (7, 8). By deleting an N-linked glycosylated asparagine at the N-1 position of RBD219, both the yield and antigenicity improved. At a 10-liter scale production process, the antigen was produced through fermentation at 400 mg/L FS with purification recovery >50% (7, 8). A panel of characterization tests indicates that the process is reproducible and robust, and that the purified, tag-free RBD219-N1 protein has high purity and a well-defined structure. It is therefore suitable for both pilot scale manufacturing and for transition into process improvements leading to industrial scale manufacturing.
2. ***Technology transfer***. The process is suitable for technology transfer to emerging market vaccine manufacturers (aka DCVMs: developing country vaccine manufacturers) having expertise in fermentation technology (https://www.dcvmn.org/) [9] and is a universally available technology in use by many DCVMs.
3. ***Shovel ready***. The antigen was manufactured under cGMP and can be vialed to produce between 20,000 and 200,000 doses, with the possibility of transferring production processes and cell banks to DCVMs for large-scale production sufficient to meet global needs.

Beyond low cost and ease of potential technology transfer to DCVMs, an advantage of employing a recombinant protein subunit vaccine is the long-standing safety record of this class of vaccines, and the fact that this technology has been used for the licensure of two other antiviral vaccines - hepatitis B and human papillomavirus, as well as biologics (e.g., insulin) [10].

Safety Evaluation of a Low-cost Recombinant Vaccine

In addition to their low cost and suitability for use in public immunization programs in low- and middle-income countries, we pursued RBD recombinant protein-based vaccines as a technology to maximize

safety relative to other platforms, such as virus vectors that have previously been found to induce immune enhancement. For instance, immune enhancement in children following a formalin-inactivated RSV vaccine was first reported in the 1960s (11) and later shown to occur in laboratory animals with early prototype SARS CoV vaccines using virus vectored platforms or inactivated virus constructs (12-15):

Avoiding Vector-platforms. Some of the earliest SARS-CoV vaccine candidates used vectored-based platforms and these were associated with immune enhancement or activation (Table 1). In 2004-05, scientists at the Public Health Agency of Canada's National Microbiology Laboratory in Winnipeg, Manitoba (which helped to develop the first successful Ebola vaccine) found that a recombinant modified vaccinia Ankara (rMVA) expressing the S-spike protein resulted in severe liver pathology upon SARS-CoV virus challenge. The major features included severe periportal and panlobular mononuclear hepatitis, together with marked ALT elevations and focal cell liver necrosis, in vaccinated ferrets following SARS-CoV challenge (13-15). The underlying mechanism of liver damage is unknown, although hepatic autoimmunity has been linked to cytotoxic T cells and T cells predominantly of the Th17 type (16). Similarly, it was reported that rMVA expressing the S-spike also resulted in lung immunopathology in rhesus macaques (17). Further, Venezuelan equine encephalitis virus replicon particles expressing SARS-CoV antigens enhanced lung pathology with eosinophilic infiltrates (18), as did recombinant vaccinia expressing nucleocapsid, membrane, envelope, and S proteins (19). In the vaccinia study, the challenged mice exhibited up-regulated Th1 cytokines and down-regulated anti-inflammatory cytokines, such as IL 10, and a mononuclear cell infiltrate, including eosinophils (19). These studies indicate that the immunopathology is a major concern for vectored vaccines, especially MVA or vaccinia, and linked to underlying mixed Th1/Th17 and Th2 responses.

Avoiding Inactivated Whole Virus Vaccines. Lung immunopathology is also linked to whole inactivated viral vaccines. For example, eosinophilic immunopathology was associated with a double inactivated SARS-CoV vaccine candidate (DIV), especially after heterologous challenge, referring to different strains of the SARS-CoV (20). However, it was determined that "eosinophilia is driven by immune responses to sequences intrinsic to the SARS nucleocapsid (N) protein" (20). Another study found that alum actually reduced DIV-induced eosinophilic histopathology in the lungs (21), while both C57BL/6 and Balb/c mice exhibited similar levels of immunopathology (21).

Table 1. Summary of Immunopathology Studies: SARS CoV Immunization and Challenge

Vaccine	Animal Model	Target Organ	Description	Reference
Vectored: rMVA expressing S protein	Ferret	Liver	Severe periportal and panlobular mononuclear hepatitis.	(13)
Vectored: rMVA expressing S protein	Ferret	Liver	Inflammatory responses and focal necrosis in liver tissue	(14)
Venezuelan equine encephalitis virus replicon particles (VRP) expressing N protein	Mice (Balb/c)	Lungs	Mononuclear leukocytes (mainly lymphocytes and plasma cells; i.e., lymphoplasmacytic cuffing) and increased numbers of widely scattered eosinophils	(18)

Vectored: rVaccinia expressing NMES proteins	Mice (Balb/c)	Lungs	Immunized mice upon infection exhibited significant up-regulation of both Th1 (IFN-gamma, IL-2) and Th2 (IL-4, IL-5) cytokines and down-regulation of anti-inflammatory cytokines (IL-10, TGF-beta), resulting in robust infiltration of neutrophils, eosinophils, and lymphocytes into the lung, as well as thickening of the alveolar epithelium	(19)
Double inactivated virus with and without alum	Mice (Balb/c)	Lungs	Using an adjuvanted and an unadjuvanted double-inactivated SARS-CoV (DIV) vaccine, we demonstrate an eosinophilic immunopathology in aged mice comparable to that seen in mice immunized with the SARS	(20)
Whole virus inactivated Recombinant S protein VLP, S, with and without alum	Mice (Balb/c) (C57Bl/6)	Lungs	Vaccines without alum had higher immunopathology scores than vaccines with alum Eosinophils Neutrophils	(21)
rMVA expressing S-protein	Rhesus Macaques	Lungs	Lung immunopathology	(17)

Recombinant Protein Receptor Binding Domain Vaccines. Given the history of virus-vector platforms and inactivated vaccines in eliciting eosinophilic immunopathology, our emphasis has been on the evaluation of inexpensive recombinant proteins produced in microbial systems, beginning with the RBD219-N1 antigen, encoding amino acids 319-536 (219 AA) of the SARS-CoV S-spike protein.

The rationale for selecting this domain of the S protein includes focusing on the key component that binds to the human ACE2 receptor, and removing the known elements of the S protein involved in immune enhancement. Evidence supporting this rationale derive from the following studies:

- Wang et al (2016) findings that peptides S597-603 outside of the RBD domain induced antibodies that enhanced infection both in vitro and in non-human primates (22).

- Jiang et al (2005) findings that the S protein truncated at amino acid 1153 did not result in immune enhancement although it did produce neutralizing antibodies. This finding suggests that removal of the aa 1153–1194 region, also outside the RBD, may halt immune enhancement (23).

- Du et al (2007) findings that RBD vaccination induced high titer of antibodies and potent SARS-CoV neutralizing activity. All except one of the vaccinated mice were protected without histopathology following virus challenge. This is in contrast to the lungs of challenged control mice (24).

Results from unpublished preclinical efficacy studies of RBD219-N1 conducted in Balb/c mice indicate that after a lethal challenge with a mouse adapted SARS-CoV, 100% of mice immunized with RBD219-N1 survived, with eosinophilia minimized by using an alum formulation (25), an adjuvant know to induce Th2 responses (26). This result is similar to what was found with DIV and other vaccines (21) and therefore contrasts the call for vaccines that induce only Th1 responses. Together these findings suggest that RBD, which can induce protective antibodies to SARS-CoV, should be considered for additional testing and its advancement into clinical evaluation.

Evaluating efficacy

There is evidence to justify advancing the RBD219-N1 antigen as either a homologous vaccine against SARS (7, 8, 12) or as a heterologous vaccine against COVID-19 (9). Against SARS CoV homologous virus challenge the vaccine formulated on alum exhibits high levels of protective immunity and with evidence of minimal immune enhancement (25). With regards to cross-protection against SARS CoV2, the RBD of the SARS-CoV-2 and SARS-CoV RBD219-N1 share significant amino acid sequence similarity (> 75% identity, >80% similarity) and recent evidence indicates that both viruses use the human angiotensin converting enzyme 2 (ACE2) receptor for cell entry (9). Further published studies indicate strong antigenic similarities between the SARS CoV and SARS CoV2 RBDs, and the potential for cross protection. For example, serum from a convalescent SARS CoV patient was shown to neutralize SARS CoV2 driven entry (27). Moreover, new studies by Tai et al (2020) find that using pseudotyped SARS CoV2, the SARS CoV RBD blocks the entry of both SARS CoV and SARS CoV2 pseudovirus into human ACE2-expressing 293T cells (28). Through pseudovirus neutralization activity, it was found that SARS CoV RBD specific antisera could neutralize SARS CoV2 pseudovirus infection, suggesting that SARS CoV RBD-specific antibodies can cross-react with SARS CoV2 RBD and cross neutralize SARS CoV2 pseudovirus infection (28). Additional studies find that multiple (but not all) neutralizing monoclonal antibodies bind to both RBDs (29-33), reviewed in Ref (9).

Next steps

An international priority is the scale-up and global access of an affordable and safe recombinant vaccine to prevent emerging coronavirus infections, including COVID-19. Our aspirational goal is to protect global populations at risk for this important emerging virus infection.

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