VACCINEINSIGHTS

COVID-19: HOW IS THE PANDEMIC CHANGING THE VACCINES SPACE?

SPOTLIGHT

Developing a protein subunit vaccine for COVID-19

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VIEWPOINT

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Vaccine Insights Editor Charlotte Barker spoke with Vikram Paradkar on April 8, 2022. This article was written based on that interview.

Supplied to the Indian government at $2 per dose, the recombinant protein subunit vaccine Corbevax has been administered to 100 million people. This article will discuss the rationale behind the choice of platform, immunogen, and adjuvants.

Biological E is one of the first biologic companies established in India and has been developing and manufacturing vaccines for more than 50 years. Pre-pandemic the company delivered more than 500 million vaccine doses per year to over 100 countries, with major products including pentavalent vaccine, DTP, TT and Td vaccines, and measles–rubella vaccines. When the COVID-19 pandemic was declared in February 2020, it was immediately apparent that only vaccines would be able to overcome this pandemic, and Biological E decided early on to focus on developing a protein subunit-based vaccine – Corbevax.

The vaccine entered Phase 1/2 trials in November 2020 and Phase 2/3 trials in June 2021. India's National Regulatory Authority granted an emergency use authorization (EUA) for adults on 28 December 2021, with EUAs for 12–18-year-olds and 5–12-year-olds granted in February and April 2022, respectively.

While mRNA and adenovirus vector vaccines were the first to be approved and have played a key role, the more traditional technology of protein subunit vaccines has important advantages. The safety profile of protein subunit vaccines is excellent, and we have seen very few adverse events, with none of the cardiovascular or blood clotting adverse events seen with mRNA and adenoviral vaccines, respectively. In three Phase 2/3 clinical trials, more than 3,500 subjects ranging in age from 5 to 80 years have received Corbevax with no reported Grade 3 or Serious Adverse Events, or adverse event of special interest. Another issue with mRNA vaccines, in particular, is that while the initial antibody response is greater than other vaccines, it wanes after a few months, requiring repeated boosters. Follow-up of clinical trial subjects receiving Corbevax indicates that good levels of immunity are preserved for at least 6 months, and possibly longer – if confirmed in larger post-marketing studies, this could be an important advantage for protein subunit formulations. Finally – and perhaps most important on a global scale – protein subunit vaccines can be manufactured at a large scale with well-established technologies, making them affordable. Biological E is supplying this vaccine to the government of India at around $2 a dose – the lowest price for a COVID-19 vaccine globally.

While protein subunit vaccines are well-established technology, every new vaccine presents challenges, and this project was no exception.

SELECTING THE IMMUNOGEN

One approach would be to use the entire spike protein as an antigen, but the SARS-CoV-2 spike protein is very large (1273 amino acids), meaning that the microbial systems the company currently uses for vaccine manufacturing would have been unable to produce the protein efficiently. We needed to find a smaller – but still immunogenic – fragment of the spike protein.

The Baylor College of Medicine and Texas Children's Hospital carried out work in 2010 on the SARS-CoV-1 virus receptor-binding domain (RBD), which binds to the ACE-2 receptor in human cells to mediate cell entry and were able to demonstrate in animal studies that it was a good vaccine candidate [1]. We established research collaborations with a number of academic labs investigating the RBD as a vaccine candidate and evaluated the nature of the protein and the recombinant microbial strains used to produce them, including conducting animal studies with several different RBD proteins [2]. Ultimately, BioE licensed the *Pichia Pastoris* strain producing the RBD of SARS-CoV-2 from Baylor College of Medicine and Texas Children's Hospital.

The RBD fragment is small and easy to handle, and we expected that this vaccine

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would be easier to develop than a complex and heavily glycosylated spike protein in terms of consistent manufacturing. As it is a small fragment of the spike protein (around 20%), we were apprehensive about whether it would generate an immune response and demonstrate protection. However, our own animal studies confirmed the earlier studies from Baylor College of Medicine, and the Adjuvanted-RBD vaccine demonstrated good neutralization of the SARS-CoV-2 virus, giving us reasonable confidence that a vaccine derived from RBD would have enough immunologically relevant epitopes. That conclusion has been borne out in the clinic, with a good immune response offered by Corbevax as well as other RBD-based vaccines such as those developed by Findlay Institute in Cuba and Anhui-Zhifei of China [3,4].

SELECTING ADJUVANTS

The selection of adjuvants is critical for protein subunit vaccines. The safety profile of the adjuvants, compatibility between antigen and adjuvants, and the reactogenicity of the resulting vaccine must all be carefully considered.

RBD as a protein is not immunogenic in itself so it must be adjuvanted for the immune system to recognize it. We evaluated various adjuvants including aluminum hydroxide (alum), one of the most common adjuvants in vaccines, squalene- and saponin-based adjuvants, and CpG 1018, an emerging oligonucleotide adjuvant used by Dynavax in their vaccine for hepatitis B. In mouse studies, each adjuvant alone was only moderately successful; however, when alum and CpG were tested in combination they gave a significant synergistic response and the desired Th1-skewed immune response to avoid antibody-mediated disease enhancement (a lingering concern for several types of vaccines). At least three other vaccines developed against COVID-19 in the same timeframe (from Clover, Medigen, and Valneva) have also chosen to adjuvant with alum and CpG, suggesting that this has been a universal finding.

MANUFACTURING & SCALE-UP

The key to scalability is consistency in the manufacturing process. It is difficult, expensive, and time-consuming to make changes at full scale. We manufacture the RBD protein antigen of Corbevax in a recombinant yeast expression system that our team has significant experience with, and which does not require complex infrastructure. This tried and trusted protein manufacturing process is the key reason we can supply large quantities of Corbevax so cost-efficiently.

Yeast expression systems inherently have good scalability and productivity, but the magnitude of the scale-up required for RBD antigen production was substantial. That led to some challenges from a logistics perspective to our current manufacturing facilities, such as the ability to supply a large quantity of oxygen to fermenters to support growth, handling of methanol required for fermentation, etc. However, with some retrofitting, these issues were quickly resolved, and we are now producing Corbevax at close to 100 million doses per month, using our existing facilities.

LOOKING AHEAD

With the data from our pediatric trials [5], Corbevax received a EUA from India's National Regulatory Authority in April 2022 that covers vaccination from age 5 years and above, and with additional clinical trials, we hope to gain approval for younger children and infants. Currently, young children do not seem to be severely affected by COVID-19, but it is impossible to predict how new variants will affect vulnerable populations. Protein subunit vaccines are routinely administered as childhood vaccinations (e.g., hepatitis B) and are proven to be safe and effective in children and infants. We are also in the process of obtaining WHO-EUL and registering the vaccine in multiple countries. Corbevax is now approved in Botswana for ages 12 years and above and is under

consideration for the 16 countries that form the Southern African Development Community. The Indian government initiated a vaccination campaign in children aged 12, 13 and 14 years with Corbevax on March 16, 2022 – to date, approximately 50 million doses of Corbevax have been administered and 15 million children have completed two-dose primary vaccination with minimal adverse events following immunization and no adverse events of special interest. This is one of the largest pediatric COVID-19 vaccination campaign worldwide.

Despite being such a small subunit protein, RBD is a vital part of the interaction of the virus with the ACE-2 receptor, and the antibodies generated appear to have significant cross-neutralizing potential for the variants already in circulation. However, there is a need to develop vaccines that can protect against variants that could emerge in the future. Future variants are not easy to predict. However, we believe that the platform that we have developed – a protein subunit plus alum and CpG adjuvants – can be adapted to develop a pan-coronavirus vaccine, provided we can develop the right antigen design. Options we are exploring include multivalent vaccines, a multi-epitope antigen, or a synthetically derived protein subunit.

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