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Innovators target vaccines for variants and shortages in global South

The next generation of COVID-19 vaccines will not only tackle viral variants but also provide solutions across the globe at a fraction of the cost.

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The world's largest vaccine maker, Serum Institute of India, is testing a new virus-like particle vaccine for COVID-19 made with technology licensed from the UK-based SpyBiotech. Credit: Reuters/Amit Dave

The one-shot Johnson & Johnson vaccine could speed global uptake and help prevent the emergence of more SARS-CoV-2 variants in vulnerable low- and middle-income countries (LMICs). This latest vaccine approved by the US Food and Drug Administration and others in the pipeline may allay global shortages that have arisen due to wealthy countries' grab for the lion's share of initial vaccine supplies. The next wave of vaccine developers is ushering in new technologies to deal with fast-spreading variants and striking deals—not with the familiar COVID-19 vaccine makers, but with producers and institutes in LMICs. The flow of technology is not all one-way, either. Bharat Biotech of Hyderabad, India, which recently reported an efficacy rate of 81% in an interim read-out from a phase 3 trial of its whole-virion inactivated vaccine Covaxin, has lined up Ocugen to act as its US development and commercial partner.

The emergence of several SARS-CoV-2 variants has led to concerns about compromised vaccine efficacy, but many vaccine developers already anticipated the issue. "The situation really isn't any great surprise to us," says Steven Powell, CEO of eTheRNA Immunotherapies, of Niel, Belgium. "It was always our intention to create a vaccine with cross-strain protection," he says. eTheRNA and other vaccine developers are adopting several different routes and modalities to build a range of options offering broad protection: from live-attenuated vaccines based on genetic reprogramming to oral vaccines, intranasal vaccines, self-amplifying RNA vaccines, computationally designed virus-like particles (VLPs), and peptide vaccines and RNA vaccines directed against T cell epitopes.

The scale of development activity is also notable. Excluding those vaccines that are already authorized, the Milken Institute's vaccine tracker lists 7 clinical-stage DNA vaccine programs, 11 inactivated virus vaccines, 1 live-attenuated virus vaccine, 8 non-replicating viral vectors, 5 replicating viral vectors, 19 subunit vaccines, 5 mRNA vaccines and 2 based on VLPs. The geographical spread of these programs is also wide (Table 1).

TABLE 1 | SELECTED VACCINES IN DEVELOPMENT FOR LOW- AND MIDDLE-INCOME COUNTRIES

Developer	Vaccine	Description	Status
Bharat Biotech, Ocugen	Covaxin (BBV152)	Whole-virion inactivated SARS-CoV-2 vaccine, formulated with Algel-IMDG adjuvant. (IMDG is an imidazoquinoline in-licensed from Virovax that acts as a TLR7/8 agonist.) The vaccine is stable at refrigeration temperature.	Phase 3
Biological E (Hyderabad,	COVID-19	Subunit vaccine comprising SARS-CoV-2 S	Phase 3

India), CEPI, Dynavax Technologies, Baylor College of Medicine	vaccine	protein RBD adjuvanted with CpG1018 plus alum	
Instituto Finlay de Vacunas (Havana, Cuba)	Finlay-FR-2 'Soberana02'	Subunit vaccine comprising SARS-CoV-2 S protein RBD conjugated to tetanus toxoid	Phase 3
Zydus Cadila (Ahmedabad, India)	ZyCoV-D	DNA vaccine encoding the SARS-CoV-2 spike protein. The vaccine is stable at 25 °C.	Phase 3
Inovio Pharmaceuticals, CEPI, Bill & Melinda Gates Foundation, Advaccine Biopharmaceuticals (Suzhou, China)	INO-4800	DNA vaccine encoding the SARS-CoV-2 spike protein, delivered by intradermal injection followed by electroporation. The vaccine is stable at room temperature.	Phase 2/3
Clover Biopharmaceuticals (Chengdu, China), Dynavax, CEPI	S-Trimer COVID-19 vaccine	Subunit vaccine comprising trimerized SARS-CoV-2 spike protein adjuvanted with CpG1018	Phase 2/3
SK Bioscience, CEPI	GBP510	Computationally designed VLP displaying 60 copies of the S-protein RBD of SARS-CoV-2, administered with an alum adjuvant	Phase 1/2
SpyBiotech, Serum Institute of India	COVID-19 VLP vaccine	HBsAg-based VLP onto which the SARS-CoV-2 S protein is attached using the engineered *S. pyogenes* CnaB2 (SpyCatcher/SpyTag) protein conjugation technology. The vaccine is stable at ambient temperature and can be lyophilized.	Phase 1/2
Codagenix, Serum Institute of India	COVI-VAC	Single-dose, intranasal, live-attenuated vaccine comprising codon-deoptimized SARS-CoV-2 genome	Phase 1
Vaxart	VXA-CoV2-1	Oral two-dose vaccine comprising two recombinant Ad5 vectors encoding the SARS-CoV-2 S and N proteins and a TLR3 agonist. Stable at room temperature.	Phase 1
Emergex (Abingdon, UK), Oswaldo Cruz Foundation	Set-point COVID-19 vaccine	Peptide vaccine directed at 11T-cell viral epitopes, delivered by microneedle injection	Preclinical

TLR, Toll-like receptor. Sources: company & organizational websites, ClinicalTrials.gov, Milken Institute, bioRxiv.org.

"One particular feature that we are focusing on is thermostability," says Nick Jackson, head of programs and technology, vaccine R&D, at Oslo-based CEPI, the Coalition of Epidemic Preparedness Innovations, which has formed the COVAX facility with the World Health Organization and GAVI to supply vaccines to LMICs. "Other vaccine types which are likely to be easier to deliver in LMICs are those which can be self-administered or more easily given—for example, a nasal spray or in a pill form. This would mean that vaccines could be more easily delivered to remote settings and less dependent on access to healthcare sites and personnel." Drone technology is also being deployed to speed distribution to remote areas—in Ghana, 2.5 million vaccines will be delivered in this way, through a partnership between Zipline, a medical drone delivery firm, the UPS Foundation and the country's government.

Even before the pandemic arrived, Tübingen, Germany-based CureVac and CEPI were co-developing portable mRNA printing technology, which could rapidly produce hundreds of thousands of vaccine doses in localized settings. "We have a 'wave 2' portfolio that we put in place last year," says Jackson. This remains at the prototype stage, Jackson says. "It really is the ticket for mRNA having a global impact in the future." CEPI is also "in active discussions" with GreenLight Biosciences, which has developed a low-cost cell-free mRNA production method that relies on harvesting nucleotides from yeast biomass.

In the meantime, new manufacturing partnerships, such as those between Basel, Switzerland-based Novartis and CureVac and between Merck and Johnson & Johnson will add desperately needed production capacity to the global supply.

The new variants are already forcing vaccine developers to retool. AstraZeneca's replication-deficient chimp adenovirus vector vaccine encoding SARS-CoV-2 spike (S) protein (AZD1222/ChAdOx1) appeared to be severely compromised in one South African efficacy trial, which prompted the country's authorities to halt its rollout. Novavax's saponin-based (Matrix-M)-adjuvanted S-protein nanoparticle vaccine NVX-CoV2373 was also impaired, though not to the same extent, according to one recent independent study. The Pfizer/BioNtech and Moderna vaccines also exhibit a major loss of neutralizing activity against the variant B.1.1.28, first identified in Brazil and Japan. Although the companies maintain that their respective modified mRNA vaccines still provide adequate protection against variants, they are developing booster shots directed at the B.1.351 variant, first identified in South Africa. Pfizer and BioNtech are also assessing the effect of a third shot in a new study, which will recruit volunteers from their original phase 1 trial. CureVac and London-based GlaxoSmithKline have also disclosed plans to co-develop a protamine-complexed unmodified mRNA-based vaccine against as yet undisclosed multivalent antigens, which they aim to introduce next year.

Further immune escape variants are likely to emerge, which for vaccine developers means broadening the range of viral epitopes they target. The first wave of vaccines converged on S protein, generating antibodies and T cells that recognize the receptor-binding domain (RBD) to prevent virus from entering the host's epithelial cells. The next-generation vaccines will need to generate immune responses that protect across strains and broaden the target viral antigens. To this end, vaccines will need to target highly conserved T cell epitopes on the virus and epitopes that elicit broadly neutralizing antibody responses. Other potential tweaks could ensure a vaccine stimulates mucosal immunity, protects with a single dose or remains stable at room temperature.

Some of these approaches are now in the clinic. The world's largest vaccine manufacturer in volume terms, the Serum Institute of India, is in a phase 1/2 trial with a COVID-19 vaccine based on VLP technology licensed from Oxford, UK-based SpyBiotech. It combines the hepatitis B virus surface antigen (HBsAg) with the SpyCatcher/SpyTag 'plug and display' protein conjugation technology to present S protein to the immune system.

HBsAg spontaneously forms a VLP, a structure to which it is possible to attach any protein antigen. The technology, developed by SpyBiotech co-founder Mark Howarth at Oxford University, exploits a domain—the second immunoglobulin-like collagen adhesin domain (CnaB2)—from the *Streptococcus pyogenes* fibronectin-binding protein FbaB, engineered into a peptide tag (SpyTag) of 13 amino acids and a protein partner (SpyCatcher) of 138 amino acids. When the SpyTag and SpyCatcher come into proximity, a stable intramolecular isopeptide bond spontaneously forms as a result of nucleophilic attack by the unprotonated amine of SpyTag's Lys31 on the carbonyl carbon of SpyCatcher's Asp117 (catalyzed by a neighboring Glu77 residue). The SpyCatcher domain can be embedded in the VLP structure and the SpyTag in any antigen of interest. Mixing the two together results in consistent, stable VLPs that are richly decorated with antigen.

"Making a VLP from scratch with a heterologous antigen does take time," says SpyBiotech co-founder, CEO and CSO Sumi Biswas. But the platform is now in place, and initial clinical data are imminent. "For the next pandemic, you do not have to make the VLP again."

Another VLP technology based on a computationally designed protein nanoparticle developed at the University of Washington's Institute for Protein Design (IPD) is in early stage trials under a partnership between SK Bioscience of Seongnam, South Korea, and CEPI, which have rights to the technology for COVID-19 vaccine development in non-Western markets. Their COVID-19 vaccine, GBP-510, comprises two protein components: a trimer protein to which the target antigen (S protein) is fused and a pentamer that, when combined with the trimer,

spontaneously forms a VLP structure. “It can be done in a very controlled way, which is very important for vaccine reproducibility,” says CEPI’s Jackson. A single particle can accommodate sixty copies of a target antigen. Mouse data show the structure to be highly immunogenic, even at low doses. “We believe the neutralizing titer of a VLP is inherently superior to soluble protein approaches,” says Adam Simpson, CEO of Icosavax, which is developing the VLP platform for multiple indications, including COVID-19. “These particles are the proper size for immune trafficking to the lymph nodes.”

A group led by the California Institute of Technology’s Pamela Bjorkman has reported promising immunogenicity data with mosaic or multivalent structures that employ the SpyCatcher/SpyTag system to decorate the IPD-developed protein nanoparticle. Containing several copies of four to eight different S-protein RBDs from various human and animal coronaviruses, these structures elicited cross-reactive immune responses in mice and provided protection against strains that were not represented in the mosaic. The effects were much stronger than those seen following immunizations with monovalent RBDs or with human convalescent plasma. “It’s not that surprising, but it did work really well,” says Caltech PhD student Alex Cohen, first author on the paper.

The group has now started testing its construct against a B.1.351 lineage strain. “From our preliminary data, ours doesn’t appear to go down very much,” says Bjorkman. “The next step will be protection studies.” Data from non-human primates will enable the group to benchmark its construct against existing vaccines. That work is getting under way shortly through a collaboration with Malcom Martin at the US National Institute of Allergy and Infectious Diseases. If this approach does yield an effective vaccine, its production would be “trivial” compared with what is entailed in the production of current protein subunit vaccines, Bjorkman says.

Live-attenuated vaccines are, like VLPs, generally highly immunogenic. What’s more, they are also expected to elicit an immune response similar to that mounted against an infection, since all of the viral antigens are present. The Serum Institute of India is also at the forefront of this approach, taking forward a live-attenuated COVID-19 vaccine through a partnership with Codagenix, which has developed codon deoptimization techniques to impair viral replication. For its SARS-CoV-2 vaccine, COVI-VAC, Codagenix has introduced 283 silent mutations into the gene encoding the viral spike protein. “Our platform is an algorithm—it’s not a carrier virus, it’s not a VLP,” says CEO and co-founder Robert Coleman. Now in phase 1 trials, COVI-VAC is administered as a single-dose, needle-free intranasal vaccine and can be easily manufactured at scale. Although the effective dose has not been established, Coleman estimates it will be in the

range of about 1 million plaque-forming units, using standard Vero cell culture production. "That's about fifty doses per milliliter," he says.

Also using a codon-deoptimized live virus is Meissa Vaccines. The company is employing the approach using respiratory syncytial virus (RSV), rather than SARS-CoV-2 itself, as a carrier vector to present the SARS-CoV-2 S protein to the immune system. Its candidate vaccine is also nasally administered in a single dose and is designed to elicit a mucosal, as well as a systemic, response. "We have the potential to block transmission and, I would say, be part of the endgame," says CEO and founder Marty Moore. A phase 1 study is due to get underway shortly. A similar construct for preventing RSV infection has already completed two phase 1 studies. "What's really unique about this vaccine is that the safety is pristine," he says. Manufacturing will also be cheap. "We're talking pennies a dose," he says.

A vaccine in tablet form would substantially ease production and distribution challenges, particularly in low-resource settings. Vaxart is among the first to take an oral COVID-19 vaccine into the clinic. "Making the vaccine is only part of the problem," says CSO and founder Sean Tucker. "The rate-limiting step will be how fast can you put it in people's arms." Preliminary phase 1 data indicate that Vaxart's vaccine, which consists of an engineered adenovirus encoding both the SARS-CoV-2 S and nucleocapsid (N) proteins, elicits a strong cytotoxic $CD8^+$ T cell response, which could provide long-term protection. The vaccine was less effective at eliciting a systemic antibody response, however.

Eliciting a strong T cell response is also the focus for Oxford, UK-based Emergex. But its approach is based on a painstaking process of identifying viral epitopes that are the target of the early T cell response. "The T cell response in convalescent blood is not the same as the T cell response you use to get rid of COVID-19," says CEO and co-founder Thomas Rademacher. Emergex is developing a synthetic peptide-based vaccine designed to generate tissue-resident T cells that recognize viral peptides generated early in the infection cycle. The company aims to begin clinical trials in the United States, Europe and Brazil, where it has a partnership with the Oswaldo Cruz Foundation of Rio de Janeiro, an established vaccine research institute.

Whether the pipeline of next-generation COVID-19 vaccines will be needed is as yet unclear, given the uncertainties surrounding both the duration of protection and the level of protection the current vaccines provide against the main pandemic strains and the emerging variants. Real-world data from those countries furthest advanced in their immunization programs will help to clear up some of the uncertainties. In the meantime, the continued flow of finance is vital to place the most promising technologies on a firmer footing. The resounding success of the mRNA vaccines was built on decades of investment—but the solution to bringing this deeply

damaging pandemic to an end is likely to require a much broader set of different vaccine platforms that work in various countries with varying resources, have different price points, work in different demographics and neutralize a broad set of viral variants.

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