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COVID-19 vaccine makers chase variant-ready vaccines

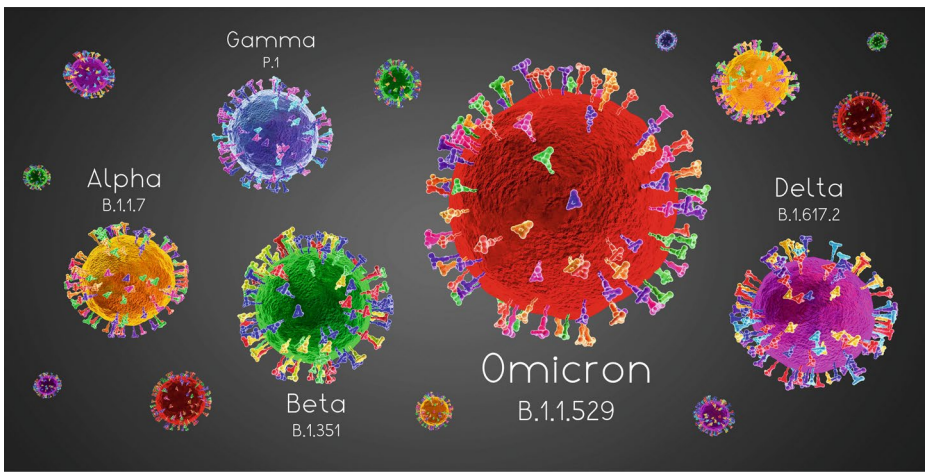
Omicron is prompting vaccine makers to look beyond SARS-CoV-2 spike protein and test whether T-cell immunity or multivalency can combat new variants

Pfizer aims to have an Omicron-specific COVID-19 vaccine ready by March. Assuming it gains approval, it will mark the first revision of its mRNA vaccine Comirnaty since the initial Emergency Use Authorization of the vaccine by the US Food and Drug Administration (FDA) on 11 December 2020. The variant-specific booster will also test the responsiveness of the FDA and other major regulators in turning around applications for vaccine revisions.

The rapid global spread of the highly transmissible Omicron variant of SARS-CoV-2 despite the first generation of vaccines demonstrates the sheer unpredictability of the pandemic. As new variants emerge, and existing COVID-19 vaccines lose their efficacy, producers and developers are switching to variant-ready vaccines and boosters; others are focusing on developing broadly protective vaccines; and yet others have vaccines to promote T-cell responses in their sights.

But even with the extraordinary speed at which mRNA and DNA vaccines are produced, the fastest turnaround time is several months, lagging behind surges of infections due to new viral variants. "By the time an Omicron variant vaccine could be made, you might have some other variant that has emerged that has a different antigenic profile," says Rick Malley, CSO and scientific founder of vaccine developer Affinivax, who also holds a chair in pediatric infectious diseases at Boston Children's Hospital. In the absence of effective multivalent vaccines that provide cross-strain protection, there is a risk that chasing variants and providing booster shots will become a default strategy.

Pfizer and Mainz, Germany-based BioNTech began working on an Omicron-specific mRNA vaccine on 25 November 2021 and stated then that, pending regulatory approval, they expected the initial batches to be available about 100 days later (April 2021). Moderna is operating on a similar timescale. "It definitely makes things easier if you have an approved product," says Franz-Werner Haas, CEO of CureVac. The Tübingen, Germany-based biotech is itself developing a second-generation mRNA vaccine, CV2CoV, which is optimized for improved antigen expression, after it obtained disappointing clinical data with its first vaccine candidate.



Vaccine developers are working to produce vaccines that offer cross-protection against new SARS-CoV-2 variants. Credit: 3D generator / Alamy Stock Photo

For mRNA vaccine makers planning variant-ready vaccines, development begins with simply changing the plasmid DNA sequence that encodes the mRNA drug substance forming the basis of the vaccine. The mRNA molecules are designed to mimic their natural counterparts: they contain a 5′ cap, which protects the molecules and ensures its stability; a 5′ untranslated region (UTR), which contains regulatory elements; an open reading frame encoding the target antigen; a 3′ UTR following the termination codon, which contains further regulatory elements; and a poly(A) tail of adenosine monophosphate nucleotides, which prevents enzymatic degradation. The in vitro transcription process and the DNase digestion of the transcribed plasmid DNA are unchanged. So too is the formulation step, in which the purified mRNA molecules are packaged into lipid nanoparticles for efficient cellular uptake. "We would typically expect that a different payload would be encapsulated and delivered with our carriers with the same efficiency as the current payload," says Tom Madden, CEO and founder of Vancouver-based Acuitas Therapeutics,

which has designed lipid nanoparticle delivery systems for both the Pfizer–BioNTech vaccine Comirnaty and CureVac's vaccine programs. "We like to think of it as a FedEx package. It doesn't matter what you put inside the package, it gets delivered to the address."

Switching to a new protein-based variant vaccine is an inescapably slower process, given the complexities associated with cultivating at scale recombinant cell lines and purifying the proteins they produce. "The speed of making an mRNA- or DNA-based vaccine will always be greater than when you have to purify a protein," Malley says. Viral-vector-based vaccines also depend on cell-based production, although the rapid development and initial approval of the chimpanzee-adenovirus-based AstraZeneca–Oxford vaccine Vaxzevria suggests that this modality could also support a relatively quick revision.

Several groups are aiming to develop products that induce cross-strain protection by including SARS-CoV-2 spike (S) protein epitopes from several variants. Although the development and manufacturing timescales are longer, the unpredictable course of the pandemic — and the apparent limitations of RNA vaccines — have put these options on a more realistic footing than was the case in mid-2021.

"I think everyone agrees that coronavirus isn't going anywhere soon," says Helen Horton, chief research officer at London-based Touchlight, which is developing a DNA-based pan-coronavirus vaccine based on its proprietary 'doggybone' DNA platform. "We already have data in large animal models suggesting that doggybone DNA can get better T-cell and antibody responses with less [drug substance] than plasmid DNA. Inherently, we're seeing greater immunogenicity," she says.

The emergence of SARS-CoV-2 immune escape variants is no great surprise — experts anticipated the problem during the early stages of the pandemic. The almost exclusive focus of the first wave of vaccine developers on S protein rapidly yielded rich dividends, in terms of effective products becoming available in large volumes within a year of the pandemic's start. But it also created vulnerabilities, as the ability of the S protein to mutate and evade neutralizing antibody responses quickly became apparent.

The administration of vaccines and S-protein-targeting therapeutic monoclonal antibodies exerts a selective pressure on escape variants. In retrospect, the high levels of immunity seen during the initial vaccine rollouts in the first half of 2021 were a transient phenomenon. "We all have to remember protection against hospitalization and death is the name of the game," says Malley. It is, he adds, "probably no longer realistic" to expect protection against symptomatic disease. Yet the existing vaccines still protect against severe disease. According to recently released data from Washington State's Department of Health, unvaccinated individuals aged 35 and over are 11 times more likely to experience hospitalization than those who are fully vaccinated; the death rate of older unvaccinated patients (65 years and over) is 15 times higher than that of older vaccinated patients.

Cellular immunity is likely driving much of this protection, but, because evaluating T-cell responses is far more cumbersome than measuring neutralizing antibody titers, supporting evidence is sparse. "The data are highly inferential, and that's what's frustrating about the field," says Andrew Allen, CEO and co-founder of Gritstone Bio. "T cells are obviously very important to viral protection and immunity, but they're poorly studied, particularly in these large vaccine trials, because it's hard."

S-protein-based vaccines presumably provoke a response from T cells, as well as B cells. The T-cell epitopes within the S protein may be more highly conserved than those recognized by antibodies — if the hypothesis that T cells account for the current protection against severe disease and death is correct. Gritstone Bio is targeting a broad array of SARS-CoV-2 proteins in its self-amplifying RNA vaccine, to elicit both an antibody response against spike and a cellular response against multiple T-cell epitopes. The company says that preliminary (pre-Omicron) phase 1 data from an ongoing UK study demonstrated cellular responses against nucleoprotein, membrane protein and open reading frame 3. According to the company's preliminary genomic analysis, the mutations found within the Omicron variant will have a minimal impact on the T-cell epitopes it had selected for inclusion: ~2% of the 146 non-S-protein epitopes are likely to be affected.

"Philosophically, we have to decide how important we think antibodies are," says Allen. Vaccine makers remain reluctant to dispense with the neutralizing antibody response against S protein, but the level of protection its offers now appears to be more limited — in terms of both the duration of protection and the breadth of coverage — than that of the cellular response. "No one's ready to pull the trigger because we haven't got complete enough data to connect those dots," says Allen. "It's rational and reasonable, I think, scientifically."

Although the emergence of Omicron has added new uncertainties to the COVID crisis, the collective capabilities of the COVID-19 vaccine developers and their myriad manufacturing partners and suppliers have increased dramatically in the past 12 months. Many supply chain bottlenecks that initially hampered vaccine production have eased, although they may never be eliminated completely. "In any complex supply chain, once you fix one bottleneck, you're simply going to identify the next one — it just moves the supply conundrum to the next limiting item in manufacture," says Madden.

Vaccine manufacturing, at its current scale of activity, is vast. The industry is estimated to have produced 11.2 billion doses of COVID-19 vaccine in 2021, according to a mid-December report from market analysts Airfinity, and, before Omicron's emergence, had been gearing up to reach 24 billion doses by mid-2022. New manufacturing innovations will introduce greater capacity, flexibility and speed to the global system. Touchlight, which is pursuing a dual COVID-19 strategy as a vaccine developer and as a contract manufacturing organization, is building a behemoth of a DNA manufacturing facility, in terms of output. Later this year, when it goes live, it will be capable of producing up to 1 kg of good manufacturing practice (GMP)-compliant DNA per month, all from a footprint of just 7,500 square feet. The company has pioneered a novel synthetic DNA species, called 'doggybone' DNA, as an alternative to plasmid DNA for mRNA vaccine makers and other customers. The versatile gene expression system, which does not contain any bacterial DNA sequences, comprises closed-capped, linear, double-strand DNA molecules. It can be produced rapidly in an in vitro GMP environment. "The ambition for Touchlight, as a company and a technology, is to supplant plasmid DNA as the predominant source of DNA for advanced therapy manufacture," says chief business officer Tommy Duncan.

CureVac, as well as developing its portfolio of mRNA vaccines, is continuing a collaboration with Tesla to create a transportable mRNA 'printer'. This consists of a self-contained, automated GMP production environment that integrates plasmid DNA production, mRNA synthesis and lipid nanoparticle formulation, with a yield of about 80,000 doses per week. When ready, it could theoretically be rapidly deployed to contain outbreaks or to cater to isolated, remote populations.

Of course, the circumstances in which these myriad technologies are being developed and tested are never ideal. Just as old generals are always fighting the last war, vaccine developers and public health officials seem to be always fighting the next pandemic — many promising vaccine technologies now in development may still be too early stage to have any impact on COVID-19. But the progress made in tackling the pandemic has still been substantial in terms of vaccine development and manufacturing. Equitable distribution is very much unfinished business, which makes the case for alternative vaccine technologies that can be deployed locally stronger than ever. ❐

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BIO PIGMENTS

Fashion's microbial dyeing machines

Our colorful garments are killing the planet's water systems. The textile industry contaminates waterways with more than 70 chemicals, including heavy metals, all in pursuit of color: it takes 200 tonnes of water to dye 1 tonne of fabric (8,000 T-shirts). Now Colorifix is introducing microbes as a natural, benign and sustainable substitute for traditional dyeing that delivers appealing aesthetics. "You look around your bathroom and see bright red stains between the shower tiles? That's bacteria secreting that color," says Jim Ajioka, co-founder of the Norwich, UK-based startup.

Fashion innovators are paying attention. Colorifix collaborated with Stella McCartney and partnered with sustainable leisurewear innovator Pangaia to produce a capsule collection. Colorifix is backed by retail giant H&M, which has plans for a product launch later this year.

Colorifix was founded in 2016 by Ajioka, a synthetic biologist at the Department of Pathology, University of Cambridge, and microbiologist Orr Yarkoni, Colorifix CEO. The two scientists traveled to Nepal initially, with a mission to develop microbial biosensors to monitor arsenic contamination in water in the Terai region. Back in Kathmandu, however, the locals pointed at the source of the problem: toxic effluents from the textile industry were polluting their waterways and impairing their health. Seeing this, Ajioka and Yarkoni pivoted to using microbes to produce environmentally friendly pigments.

"First we find a color we are interested in, and the genes responsible for making that color," says Ajioka, by trawling through open genomic databases of animals, plants, insects and microbes. The scientists then design a genetic pathway, synthesize the DNA and introduce it into microbes such as *Escherichia coli* or *Pseudomonas* to produce the color. Scaling up to make more pigment takes growing more bacteria in closed fermentation vessels, which prevent spillage. Once in the dyeing machine, because microbes readily adhere to the fabric and deposit the dye, the fabric coming out needs one rinse only and no fixation, unlike petrochemical-derived pigments that require arsenic-based fixatives. With the Colorifix process, the textile is exposed to heat to inactivate the microbes and finish the process, saving, according to an independent life cycle analysis, 50% in water and electricity usage and cutting CO_2 emissions by half compared with traditional dyeing.

Credit: Colorifix

Colorifix has raised a $22 million series B led by H&M with participation from SynBioVen, The Mills Fabrica, Sagana and Cambridge Enterprise.

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