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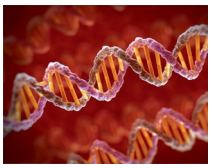

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First COVID-19 DNA vaccine approved, others in hot pursuit

India's approval of a nucleic acid vaccine hints at a solution for low-income nations—if the limitations of current delivery technology can be overcome.

The Emergency Use Authorization of a DNA-based COVID-19 vaccine by India's regulator is a milestone for a nucleic acid technology that has been largely overlooked during the pandemic. Although the approval of ZyCoV-D from Indian pharma Zydus Cadila represents a historic first for DNA-based vaccines, peer-reviewed data describing the safety and efficacy of the spike-protein-encoding vaccine have yet to be published. If DNA vaccines can overcome historic inefficiencies of delivery to antigen-presenting cells (APCs) and concerns can be allayed as to potential genotoxicity risks that could arise from chromosomal integration, their high stability, durability of response (including enhanced T-cell immunity) and ease of manufacture could make them a valuable alternative to mRNA, adenoviral vector and recombinant protein vaccine technologies. With several other DNA vaccines for COVID-19 in the pipeline (Table 1), this modality may ultimately offer a new tool for global immunization efforts in low-income countries.

India's ZyCoV-D vaccine uses circular DNA to protect against SARS-CoV-2 infection. Credit: Zydus Cadila

The COVID-19 pandemic has been an extraordinary proving ground for mRNA-based vaccines, but whether it will be so for DNA vaccines as well is unclear. "There still is a technological hurdle—and that's delivery," says Connie Schmaljohn, director of the US National Institute of Allergy and Infectious Diseases' Integrated Research Facility at Fort Detrick. Schmaljohn has extensive experience in DNA vaccine development from her previous role as a senior scientist in the US Army. A DNA vaccine needs to deliver its genetic payload to an antigen-presenting cell, just like an mRNA vaccine, but within the cell, the final destination differs. "It's easy enough to get it [RNA] into the cytoplasm, but then you have that added step with DNA—you have to get it into the nucleus," Schmaljohn explains.

Interest in DNA- and RNA-based vaccines dates back three decades. In 1990, scientists showed that naked nucleic acids injected into mouse muscle could stimulate protein production, and shortly after, another group immunized mice using a gene gun to deliver plasmid DNA directly to the skin. The mice responded by mounting an antibody response against the expressed proteins.

Mainly because RNA's instability and inflammatory potential are much greater than those of its deoxyribose counterpart, the initial focus of researchers was on DNA vaccines. Long before BioNTech and mRNA vaccine development, Pfizer was investing in a DNA vaccine platform. In 2006, the New York–based pharma acquired PowderMed, a UK firm whose predecessor, PowderJect Pharmaceuticals, had developed a gene gun that used pressurized helium to propel DNA-coated microscopic gold particles into APCs present in the skin for vaccination. Only later, after the work of Katalin Karikó and Drew Weissman showed that RNA's shortcomings could be overcome by introducing nucleoside modifications, did interest reignite in mRNA vaccines.

The shift to mRNA accelerated once the initial promise of DNA vaccines was not realized. For PowderMed, according to Schmaljohn, a major stumbling block was the difficulty of packaging a DNA vaccine into user-friendly delivery device. And expectations were perhaps unreasonably high. "Everybody was looking for home runs for the hardest possible diseases," says Margaret Liu, chairman of the board

Table 1 | Selected DNA-based COVID-19 vaccines in development

Developer	Vaccine	Description	Status
Zydus Cadila; Department of Biotechnology, Government of India	ZyCoV-D	Three-dose DNA plasmid vaccine encoding SARS-CoV-2 S protein and IgE signal peptide delivered intradermally by the needle-free PharmaJet Tropis device	Received Emergency Use Authorization in India, 20 August 2021
AnGes, Osaka University, Japan Agency for Medical Research and Development	AG0302-COVID19	Two-dose DNA plasmid vaccine encoding SARS-CoV-2 S protein delivered by intramuscular injection	Phase 2/3
Inovio Pharmaceuticals, Advaccine Biopharmaceuticals (Suzhou, China)	INO-4800	Two-dose sequence-optimized DNA plasmid vaccine encoding a SARS-CoV-2 S protein, delivered by intradermal injection followed by electroporation at the injection site with the Cellectra 2000 device	Phase 2/3
Genexine	GX-19N	Two-dose DNA plasmid vaccine encoding SARS-CoV-2 S and N proteins	Phase 2/3
Entos Pharmaceuticals	Covigenix VAX-001	Two-dose proteolipid-vehicle-formulated DNA vaccine encoding the SARS-CoV-2 S protein and two genetic adjuvants, delivered by intramuscular injection	Phase 1/2
Rottapharm Biotech (Monza, Italy), Takis (Rome)	COVID-eVax	Two-dose DNA plasmid vaccine encoding the receptor-binding domain of the SARS-CoV-2 S protein, delivered by intramuscular injection followed by electroporation	Phase 1/2
Scancell (Nottingham, UK)	Covidity	Four-dose DNA vaccine regimen, delivered by intradermal and intramuscular injection with a PharmaJet needle-free device, comprising two doses each of SCOV1 and SCOV2 plasmid DNA, which encode the receptor-binding domain of the SARS-CoV-2 S and N proteins expressed by the original pandemic strain and by a variant strain, respectively.	Phase 1
Symvivo	bacTRL-Spike	Single oral dose of *Bifidobacterium longum* bacteria engineered to deliver plasmid DNA encoding SARS-CoV-2 S protein	Phase 1

Sources: Company websites; ClinicalTrials.gov; PubMed

of the International Society for Vaccines, who, during a decade at Merck Research Laboratories, became one of the early pioneers of DNA vaccine development. "TB, malaria and HIV were the main targets—and nobody has really solved those really well," she says.

So far, DNA vaccines' only successes have been in veterinary medicine. An equine vaccine for West Nile virus was licensed by the US Department of Agriculture's Center for Veterinary Biologics in 2005; a canine vaccine against tyrosinase for melanoma in 2010; and two salmon vaccines, for hematopoietic necrosis virus in 2005 and salmon alphavirus subtype 3 in 2016.

This makes the green light from the Drugs Controller General of India to Zydus Cadila, of Ahmedabad, India, all the more noteworthy. As yet, the company's production capacity remains modest. At full capacity, the company aims to produce 100–120 million doses annually. But because ZyCoV-D is encoded in pVAX-1, a standard, commercially available plasmid vector, and is produced with a generic process, it could be readily manufactured by third parties as well. "There's no secret sauce in terms of how they made it," Liu notes.

As *Nature Biotechnology* went to press, the company had yet to publish the data from the phase 3 trial that secured the vaccine's approval. So far, it has stated in a press release that the vaccine is 67% effective at preventing symptomatic infection and 100% effective at preventing moderate disease. Although the two approved mRNA vaccines for COVID-19 were ~95% effective at preventing symptomatic infection, direct comparisons with the Zydus Cadila trial are not valid, according to Liu. "They [Zydus Cadila] were in a much tougher setting, as the Delta variant hadn't emerged when Pfizer and Moderna did their studies," she says. Vaccine effectiveness is, in any case, a moving target. It can vary considerably over time and across different studies, as the ongoing forest plot analyses compiled by the International Vaccine Access Center, at the Johns Hopkins Bloomberg School of Public Health, and the World Health Organization (WHO) demonstrate.

> "DNA-based vaccines are clearly less potent than mRNA vaccines. They need higher doses to stimulate an immune response against SARS-CoV-2."

At the same time, DNA-based vaccines are clearly less potent than mRNA vaccines. They need higher doses to stimulate an immune response against SARS-CoV-2. For instance, Comirnaty (BNT162b2), the mRNA vaccine developed by Pfizer and BioNTech, comprises two doses, each of 30 micrograms, and Spikevax, the Moderna vaccine, involves two 100-microgram doses. In contrast, ZyCoV-D is reportedly (official documents on the product were not available at the time of writing) a three-dose regimen, each containing 2 milligrams of plasmid DNA encoding the SARS-CoV-2 spike (S) protein and an immunoglobulin E (IgE) signal sequence to promote secretion. The large dose disparity between the two modalities is most likely due to the higher delivery hurdle that DNA must clear before it can start producing antigen.

The current mRNA vaccines follow a well-known delivery route. Lipid nanoparticles carrying the mRNA are taken up by APCs by endocytosis. From the endosome the mRNA escapes into the cytoplasm, most likely through electrostatic interactions between positively charged lipids and negatively charged endosomal membranes. In contrast, DNA vaccine delivery to the cell's nucleus is not a settled science. It is not yet known where

the plasmid DNA injected into the skin collects—it could be in APCs like dendritic cells, in fibroblasts, in dermis or in muscle myocytes, or all of the above. Regardless of their cellular destination, to function, DNA plasmid-based vaccines need to reach the nucleus. There they are transcribed into mRNA, which then exits the nucleus to undergo translation in the cytoplasm. Critics have raised theoretical concerns about the integration of plasmid DNA into the genome of recipient cells, but there has been no experimental evidence of this, despite, as Liu notes in a recent review, extensive testing of the first licensed animal vaccines.

So far, scientists have tested lipid nanoparticles, electroporation, jet injectors and gene guns with varying success, and new delivery technologies continue to emerge. The ZyCoV-D vaccine uses PharmaJet's needle-free Tropis device. It employs a pressurized jet of liquid, powered by a simple spring mechanism, to puncture the skin and deliver the vaccine intradermally. It is already pre-qualified under the WHO's Performance, Quality and Safety assessment process, which means it can be readily deployed by United Nations agencies and WHO member states. At a reported price of $3.57 per dose, the vaccine is not particularly cheap—a full course will cost over $10.

> "My personal opinion is we're never going to get to mass vaccination with DNA until we find a way to deliver it that's less device dependent."

Most of the cost is down to the liquid injection device. Electroporation is another tried and tested way to boost the cellular uptake by applying an electric field to the injection site, causing transient pores to form in the cell membrane and allowing large molecules to pass across. However, progress with this technology has suffered some setbacks. Inovio Pharmaceuticals, a leader in the field, had the phase 3 trial of its DNA-based COVID-19 vaccine INO-4800 put under a partial FDA clinical hold because of outstanding questions about its Cellectra 2000 handheld device, which uses electroporation. The company has since obtained authorizations to conduct the phase 3 portion of its ongoing pivotal trial in Brazil, the Philippines, Mexico and Colombia, but even if the vaccine does reach the market, scaling up could be a challenge. "I think it's always problematic for global distribution when you have a device that's high tech," says Liu. Schmaljohn concurs: "My personal opinion is we're never going to get to mass vaccination with DNA until we find a way to deliver it that's less device dependent," she says. It's the main reason why the US Department of Defense discontinued funding of several DNA vaccine programs. "A lot of places, including the Department of Defense, were very hesitant to be device dependent, whether it be electroporation or gene gun."

An efficient, ultra-low-cost device could help to shift that mindset. A group at the Georgia Institute of Technology, Emory University and Sun-yat Sen University, in Shenzhen, China, has developed an electroporation device for SARS-CoV-2 vaccination that costs less than a dollar to produce. It relies on a piezoelectric element—a thumb-operated trigger like that in a domestic stove lighter—to generate a very short electric pulse of 10 microseconds. "Electroporation is usually done with a much longer pulse," says Georgia Tech's Mark Prausnitz, one of the corresponding authors on a recent publication that describes the device. But the group was able to generate an electric field that was strong enough to ensure efficient gene uptake by designing a tightly spaced array of microneedle electrodes to deliver the current to the skin. In mice, the system was about ten times more efficient than conventional intramuscular or intradermal injection at eliciting a neutralizing antibody response against a SARS-CoV-2 DNA vaccine. It is completely portable, weighing less than 50 grams, and involves none of the cost or power requirements of conventional electroporators. "I think there's a new appreciation that cost matters in healthcare," Prausnitz says. Clinical trials are still some time away, however.

Entos Pharmaceuticals, of Edmonton, Alberta, is starting a phase 2 trial in South Africa of a DNA-based COVID-19 vaccine that employs device-free delivery. Its Fusogenix technology combines naturally occurring lipids with a non-immunogenic membrane fusion protein derived from a reovirus. These proteolipid nanoparticles fuse directly with the recipient cell membrane and deposit their contents directly into the cytoplasm, bypassing the endocytic pathway that lipid nanoparticles use. "For our platform that's a huge distinguishing feature," says CEO and co-founder John Lewis. "We're able to achieve very, very high dosing with great systemic tolerability. They go pretty much everywhere," says Lewis. The Fusogenix technology has the advantage of being fridge stable and, "because of the kinetics of expression, we actually have pretty good data showing it's probably effective after a single dose," Lewis adds. Vaccines based on mRNA

Moderna feud with NIH over COVID vaccine

A disagreement over who owns the patent rights to a landmark COVID-19 vaccine spilled into public view last month as Moderna and the US National Institutes of Health (NIH) pressed their claims. The feud stems from a four-year collaboration on HIV and emerging infectious diseases in which three scientists at NIH's Vaccine Research Center—director John Mascola; Barney Graham, who recently retired; and Kizzmekia Corbett, now at Harvard—worked with Moderna to design the genetic sequence that prompts the vaccine to produce an immune response. Results from this collaboration played "a major role in the development of the vaccine," according to NIH director Francis Collins. "It's not a good idea to file a patent when you leave out important inventors, and so this is going to get sorted as people look harder at this," Collins added. In addition, Moderna received nearly $10 billion in US government funding for large-scale clinical trials and to increase manufacturing and delivery of vaccines. Moderna countered, with a spokesperson saying the company "all along recognized the substantial role that the NIH has played in developing Moderna's COVID-19 vaccine." But in a separate statement, the company said, "We do not agree that NIAID scientists co-invented claims to the mRNA-1273 sequence itself. Only Moderna's scientists came up with the sequence for the mRNA used in our vaccine." While the NIH has traditionally declined to exercise its march-in rights on technology it has funded, co-ownership of the vaccine's patent rights would allow the US government greater say in allowing out-licensing of the technology and manufacturing rights, as well as a piece of the projected $18 billion this year—and $22 billion in 2022—that Moderna stands to earn with its only approved product.

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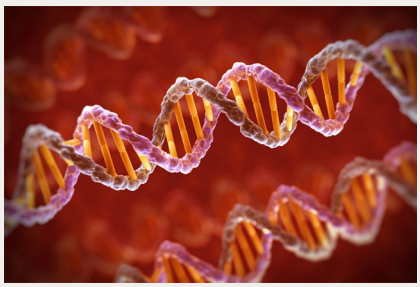
100,000 whole-genome sequences' diagnostic bonus



Sequencing as a diagnostic tool. Credit: Science Picture Co / Alamy Stock Photo

Early data from the 100,000 Genomes Project reveal to what extent patients with undiagnosed rare diseases can end their diagnostic odysseys by having their whole genomes sequenced. For the pilot study, published in the *New England Journal of Medicine*, the UK National Health Service (NHS) collected data from 4,660 people from 2,183 families with a broad swath of rare diseases. The pilot's analysis of the pioneering project, run by Genomics England, revealed that a quarter of patients with rare diseases received a new diagnosis. Of these, 14% included genome variants and coding variants in regions that would have been missed by exome sequencing and other genetic tests. For the study, the researchers collected clinical features data with Human Phenotype Ontology terms, along with genome sequences obtained with HiSeq 2500 Illumina instruments. "The key to the successful diagnosis of the patients reported in this study is the design of the analysis, where the investigators integrated the clinical features of the disease with the genome sequencing data," says Bridget Bax, Reader in Rare Diseases, St George's, University of London.

Genomics England, wholly owned by the Department of Health and Social Care, was set up in 2013 to deliver the 100,000 Genomes Project. The pilot analysis shows that in 25% of those who received a diagnosis, physicians could make immediate clinical decisions tailored to the patient's condition. The researchers hope that their findings will help other health systems consider the use of genome sequencing to care for patients with rare disease. The genomic and clinical data from the pilot study are freely accessible to members of a Genomics England Clinical Interpretation Partnership.

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rapidly produce a large spike of antigen, but they are quickly eliminated. DNA vaccines produce antigen more slowly, but over a longer time frame. "We're expressing antigen for one to two months," Lewis says.

To kick-start antigen production, the DNA plasmid must reach the nucleus from the cytoplasm, where it is delivered. The process is not fully controlled, but certain design considerations can help. "All modern plasmids have sequences optimized to get into the nucleus," Lewis says. Small size is another important parameter that can help maximize diffusion, he adds. Lindy Durrant, CEO of Nottingham, UK-based Scancell, says rapid release of the plasmid from its carrier vehicle is also essential. "If you can deliver into the cytoplasm and get rapid release of the DNA, I don't think access to the nucleus is an issue," she says.

Targeting APCs is, for most DNA vaccine developers, not a controlled process either. "Some of that is still kind of a mystery," says Schmaljohn. In gene gun studies, she says, Langerhans cells, a population of tissue-resident macrophages in the skin, were responsible for taking up and expressing plasmid DNA before migrating to the lymph nodes. Muscle tissue, in contrast, does not contain APCs. "Nobody actually has a clear idea why intramuscular injection works." The administration itself may kick-start the immune response. "There is inflammation associated with the injection, so you do get antigen-presenting cells showing up," says Lewis. Scancell aims to boost $CD8^+$ T-cell responses to its DNA vaccine Covidity by including a targeting mechanism that improves cross-presentation of extracellular antigens. The vaccine comprises two plasmids, each of which encodes the S protein receptor-binding domain and the nucleocapsid (N) protein from a particular SARS-CoV-2 strain. APCs that take up and express the plasmids can present the associated epitopes directly, but, if secreted, the N antigen is designed to be taken up preferentially by dendritic cells because of the addition of a modified IgG1 crystallizable fragment (Fc) domain, which recognizes the high-affinity Fcγ receptor they express on their surface. Previous work in cancer indicates that the approach elicits high-avidity $CD8^+$ T cells and memory T cells. "Although neutralizing antibodies are quite good, long-term protection requires T cells," Durrant says.

The current growth in COVID-19 cases in wealthy countries that have already attained high levels of vaccination is a function of both waning immunity and the mismatch between the first wave of approved adenoviral vectored, inactivated and modified mRNA vaccines and the now-dominant Delta variant. It remains an open question whether DNA vaccines will provide more durable protection than their mRNA counterparts or any other type of vaccine. Even so, the urgent need for a broad set of safe, effective and affordable vaccines—with the flexibility to deal with emerging variants—is adding increased impetus to the development of this recently neglected vaccine modality, with its unique safety and efficacy profile. ❐

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