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Researchers at Sinovac Biotech in Beijing are working on a vaccine for SARS-CoV-2.

COVID-19 VACCINES GET BIOTECH BOOST

Advances in vaccine technology are accelerating the race to stop the coronavirus — and other pathogens, too. **By Amber Dance**

In January, Barney Graham had a new vaccine ready for testing. Its target was the Nipah virus, which had caused respiratory illness and brain infections in past outbreaks in southeast Asia. Graham, a vaccinologist and deputy director at the US National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center in Bethesda, Maryland, was working with Moderna Therapeutics in Cambridge, Massachusetts, to build a type of vaccine never before approved for use. Unlike most vaccines, which are based on intact pathogens or their structural components, Moderna's are built from a pathogen's RNA. The team hoped that a vaccinated person's cells would use the RNA to make protein, priming their immune system to generate a protective response.

Graham and his colleagues were just about to start manufacturing the Nipah vaccine for human trials when they got wind of a disease caused by a new coronavirus, now known as SARS-CoV-2, wreaking havoc in Wuhan, China. They quickly changed their plans, but not the design on which their vaccine was based. Armed with the draft genome for SARS-CoV-2, which was shared online on 11 January, Moderna swapped in the coronavirus RNA and started shipping a potential vaccine to the NIAID for clinical tests. The process took just six weeks — the fastest turnaround from project start to vaccine candidate in medical history.

Graham's team is one of more than 150 vaccine developers racing against time to develop vaccines that reduce the severity of COVID-19, or block infection by SARS-CoV-2. Thanks to a wave of new vaccine technologies, these scientists stand a better chance of success than against any previous new virus. "The real challenge is speed," says Drew Weissman, an immunologist at the University of Pennsylvania in Philadelphia. However, he adds, it should not come at the expense of safety or efficacy — whether it protects humans from getting the disease.

Today, approaches such as RNA vaccines are helping researchers to create and test vaccine candidates at breakneck speed. Anthony Fauci, director of the NIAID, told a congressional committee on 23 June that he is "cautiously optimistic" that there will be a working vaccine by early 2021. Vaccinologists who spoke to *Nature* echo that sentiment — although they say that the first vaccines might not be the best possible designs, and improved versions are likely to come later. Many of the vaccines already in development have potential to become those second-generation vaccines. Several groups are applying structure- and computer-based analyses of the interactions between the immune system and viral antigens, the parts of viruses that provoke an immune response. These techniques have already been tried against numerous pathogens, and are now being applied to SARS-CoV-2.

Poised and ready

This is the moment that vaccinologists have been preparing for. Following a large outbreak of Ebola virus in West Africa in 2014–16, the World Health Organization published a short list of pathogens thought most likely to cause severe disease outbreaks in the future (see go.nature.com/2deknbt). It includes the coronavirus that causes severe acute respiratory syndrome (SARS-CoV); the related coronavirus MERS-CoV, the cause of Middle East respiratory syndrome; and a placeholder for some as-yet-unknown threat. In 2017, the Coalition for Epidemic Preparedness Innovations (CEPI) launched a global effort to unite public, private, philanthropic and civil-society organizations to develop vaccines to prevent future epidemics.

Although SARS-CoV-2 is new, researchers already knew a lot about coronaviruses in general, and learnt much from vaccine studies started during the SARS and MERS outbreaks in 2003 and 2012. One promising candidate antigen for a SARS-CoV-2 vaccine is known as the spike protein, which sticks out from the virus to create the 'crown' appearance that gives coronavirus its name. The spike protein latches on to cells and 'unlocks' them for virus entry; an antibody that binds to the spike and prevents the virus from getting into cells should stop the virus from causing disease. Such 'neutralizing antibodies' are the goal of many vaccines. Also desirable are vaccines that can induce the

production of T cells, a type of immune cell needed to produce a full immune response.

Driving these efforts are large infusions of public and private funding. CEPI estimates that it will cost US$2 billion to create the vaccine it is developing with the pharmaceutical giant GlaxoSmithKline, based in London; it has already raised more than half that amount. CEPI has also invested hundreds of millions of dollars in other partners, including AstraZeneca in Cambridge, UK, and Novavax in Gaithersburg, Maryland. The US Biomedical Advanced Research and Development Authority has poured around $3.8 billion into vaccine development by companies that include Moderna, Merck and Janssen, as well as AstraZeneca and Novavax.

That money is needed for developing a vaccine and putting it through clinical trials, as well as for manufacturing enough of a successful vaccine for use. "It's one thing to show it works, another thing to say you can supply it," says John Shiver, head of vaccine research and development at Sanofi Pasteur in Swiftwater, Pennsylvania. Sanofi plans to repurpose its influenza-vaccine facilities, which are needed only seasonally, to produce the protein-based SARS-CoV-2 vaccine it is developing. Other companies are still learning how to safely make the new kinds of vaccine, such as those containing RNA, at large scale.

Working faster

Early vaccines against viruses were based on weakened, killed, or live but harmless versions of the disease-causing agent. These strategies are still used today, as are approaches that use isolated proteins or carbohydrates as antigens.

For these kinds of vaccine, researchers more or less start from the drawing board for each new pathogen. By contrast, many vaccines in development rely on 'platform technologies', which are essentially 'plug-and-play'. For these, researchers identify what they think might be an effective antigen and drop its DNA or RNA sequence into a pre-validated platform — such as another virus genome or a piece of DNA or RNA — to quickly create a vaccine candidate. "This is ideal for emerging-pathogen vaccine development," says Sarah Gilbert, a vaccinologist at the University of Oxford, UK. Yet because they are relatively new, these platforms have yielded no licensed products for human use — and only a handful of DNA-based vaccines for veterinary use, such as for West Nile virus in horses. "There's nothing which has really gone to the people on a mass scale," says Shashank Tripathi, a virologist at the Indian Institute of Science in Bangalore. COVID-19 could provide their big debut, setting the stage for platform vaccines against other human diseases.

Moderna's vaccines are based on messenger RNAs that instruct cells to make protein antigens. The idea is that, once a person receives those RNA instructions in an injected vaccine, their cells can start pumping out the proteins. These are then displayed on cell surfaces or released into the circulation, where they can grab the attention of the immune system.

Weissman, who co-developed the technology that Moderna uses, has already built candidate RNA vaccines against influenza virus[1], herpes simplex virus 2 (HSV-2; ref. 2) and HIV[3], and expects to launch human trials of these within a year. In animal studies, his team's RNA vaccine for HSV-2 outperformed a protein-based vaccine[2]. "When we use RNA, it works better," he says. In part, that could be because the RNA lasts for about two weeks in cells, he says, giving the immune system time to detect the antigens and build immunity.

> **"It's one thing to show it works, another thing to say you can supply it."**

In a phase I safety trial, Moderna's mRNA vaccine for SARS-CoV-2 was well tolerated by people[4], and a phase II study has begun to determine the dose required. A phase III trial, designed to assess the vaccine's efficacy is scheduled to start this month.

Yet for all its apparent simplicity, the technology presents new challenges for vaccine developers, such as how to make large quantities of medical-grade RNA. CureVac in Tübingen, Germany, which is also developing RNA vaccines, has worked out how to do that, says its chief technology officer Mariola Fotin-Mleczek. RNA is naturally unstable and needs to be stored frozen at below −20 °C, which will complicate the shipping and clinical use of RNA vaccines. But CureVac and other companies are working on stabilizing the molecule at higher temperatures, for example, by freeze-drying.

DNA is more stable: it can stay intact for a year at room temperature, and longer in the refrigerator, says David Weiner, a molecular immunologist and executive vice-president at the Wistar Institute in Philadelphia, who is developing DNA vaccines. These work on the same principle as RNA vaccines, with the vaccine DNA encoding a protein antigen. When inserted into cells, the DNA is transcribed into mRNA, providing instructions for making the viral protein. Weiner has already tested DNA vaccines in people against HIV and the Ebola, MERS and Zika viruses. His Zika vaccine[5], which took 6.5 months from inception to human testing, held the previous record for fastest development.

Inovio Pharmaceuticals in Plymouth Meeting, Pennsylvania, which has licensed Weiner's technology, reported on 30 June that its DNA-based vaccine against SARS-CoV-2 was safe in people tested in a phase I trial (go.nature.com/3jeyjyd). In addition, most of the participants mounted an immune response that consisted of antibodies and T cells. A phase II/III trial to test efficacy and dose is planned for later in the year.

The technology to make large quantities of a specific DNA segment is available, says Weiner, thanks to pre-existing gene therapies, the veterinary DNA vaccines and an immunotherapy for human papillomavirus that is now in a phase III clinical trial. The challenge with DNA is delivery: the genetic material must get across the cell membrane and into the nucleus. Typically, an electric pulse to the skin is used to open up cell membranes, which Weiner says isn't quite as bad as it sounds. "It is well tolerated."

In Oxford, Gilbert has her own platform, called ChAdOx1, which is based on a chimpanzee adenovirus. Using the adenovirus genome as a carrier, the Oxford team adds the DNA sequence encoding an antigen — for SARS-CoV-2, that would be the spike protein — and injects the resulting virus particles into the recipients. The adenovirus rouses the immune system, and infected cells display the spike protein on their surfaces. Because this adenovirus does not replicate, a high dose is required, producing about 12 hours of flu-like symptoms as the immune system gears up. But any infected cells are eventually gobbled up by immune cells, so it's "completely safe", Gilbert says. Related vaccines, such as one against Ebola using the vesicular stomatitis virus as a vehicle, can copy themselves, which increases the risk of side effects or illness due to the vehicle.

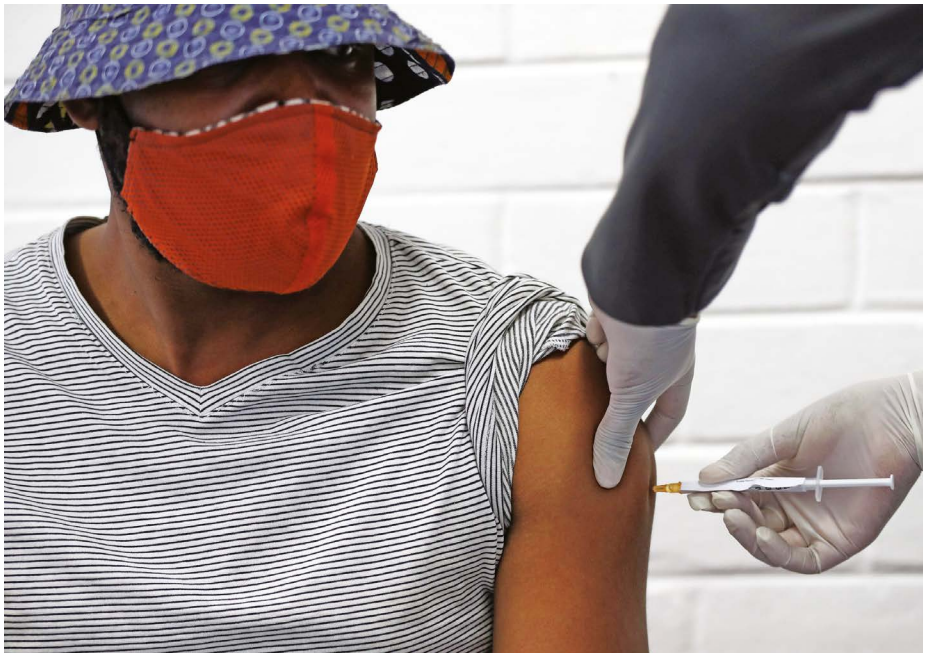
The Oxford group has tested ChAdOx1 vaccines against MERS, flu and tuberculosis in small-scale human trials, and is working with AstraZeneca to produce a version against SARS-CoV-2. That has already entered phase II/III trials, which will determine whether it protects people from the disease and whether one dose or two is more effective.

Working smarter

As the first generation of SARS-CoV-2 vaccines enters clinical trials, researchers are hard at work developing second-generation designs, incorporating all they've learnt from vaccine development for other viruses.

For example, HIV continually mutates, making it hard for the immune system to develop broadly neutralizing antibodies that can stop all the different versions of the virus. In a technology dubbed 'antibodyomics', researchers have applied informatics to analyse the characteristics and development of broadly neutralizing antibodies in the hope of designing more effective vaccines[6].

On the antigen side, researchers in Brazil have developed an approach they call 'immunoinformatics' to identify potential



SIPHIWE SIBEKO/AFP/GETTY

A volunteer in Soweto, South Africa, is injected with a SARS-CoV-2 vaccine in a clinical trial.

vaccine candidates. Alex Reis, a parasitologist at the Federal University of Ouro Preto, and immunoparasitologist Rory Brito, a postdoc in his lab, applied the technique to visceral leishmaniasis. This disease is caused by the protozoan parasite *Leishmania* that infects people and dogs, particularly in South America and in the Mediterranean region.

Reis has already developed a candidate leishmaniasis vaccine, based on broken-up parasite cells, that is going into phase III trials. But its production requires growing large quantities of the parasite, and he thinks he can make something more effective using individual peptides from *Leishmania* antigens.

Reis and Brito want to encourage T cells to fight the pathogen. To start, Brito trained computer algorithms to identify peptides in parasite antigens that are predicted to be best at stimulating a T-cell response[7]. Then, using computational analyses and animal studies, he narrowed the parasite's more than 8,000 proteins down to four peptides he predicted should be effective. In mice injected with those four peptides, and later injected with *Leishmania* parasites, the cocktail activated T-cell immunity and reduced the number of parasites, compared with unvaccinated animals[8]. Reis and Brito have yet to compare an immunoinformatics candidate against Reis's original leishmaniasis vaccine.

Using informatics, "you can propose candidate vaccines in reduced time," says Brito, "and the cost should be lower". Immunoinformatics should work for any pathogen, he adds, and the pair are applying for funding to try their approach on SARS-CoV-2. They hope to identify antigens, perhaps away from the spike protein, that could generate immunity against this and other coronaviruses.

Also influencing vaccine development are studies of pathogen protein structure. For example, Graham and his collaborators have applied structure-based design to respiratory syncytial virus (RSV), which can cause serious airway and lung infections in infants and older adults. It has resisted attempts to make a vaccine against it for decades.

Neutralizing antibodies for RSV tend to bind to a 'pre-fusion' version of a viral protein the pathogen uses to enter cells. In 2013, the team determined the structure of that pre-fusion version in a complex with neutralizing antibodies[9]. The researchers then tinkered with its amino acids to lock it in that shape[10]. A vaccine made from that version, they reasoned, would be more likely to create such antibodies. In a human trial, their vaccine boosted the neutralizing activity present in the blood more than other RSV vaccines did, and even more than the virus itself[11].

"You can propose candidate vaccines in reduced time, and the cost should be lower."

Researchers are now applying the same approach to SARS-CoV-2, using stabilized spike-protein structures in the hope of creating a more powerful vaccine[12,13].

"I think structure is probably going to be part of every vaccine-development programme that ever comes in the future," Graham says.

Leapfrogging to the finish line

As COVID-19 vaccines proliferate, the next hurdle is testing. It can take a decade or longer to go from preclinical research to production. With tens of millions of people already infected and more than half a million dead and counting, the world cannot wait. But, as researchers learnt during the Ebola outbreak, running different stages of vaccine research and testing in parallel, instead of taking the conventional step-by-step approach, can speed up development. "Those barriers have been kind of smashed down," says Helen Petousis-Harris, a vaccinologist at the University of Auckland, New Zealand.

In addition, vaccinologists plan to accelerate testing by running trials on large groups of people in hotspots where SARS-CoV-2 is spreading rapidly. That way, any differences in infection rates between vaccine and placebo groups should emerge quickly, says Weissman.

One vaccine based on an adenovirus platform — made by CanSino Biologics in Tianjin, China — received approval as a 'specially needed drug' by the Chinese military on 25 June, after unpublished phase II trial results suggested the vaccine "has potential to prevent diseases caused by SARS-CoV-2", the company reported.

Once scientists have found a vaccine that works, the challenge shifts to manufacturing. "We are talking about billions of doses needed, and this is really difficult," says Fotin-Mleczek. She notes that similar quantities of sterile needles and other equipment will probably be required, too.

Drug makers are manufacturing vaccines now, in the hope of distributing them widely as soon as they know they work. "There's not a guaranteed return on investment," says Petousis-Harris, but if a vaccine works, "you've got a huge head start."

By next year, vaccinologists expect to have not only working COVID-19 vaccines, but also a slew of new knowledge about their development. "It's going to be really interesting to compare these technologies head to head," says Gilbert. "We'll be in a much better place for the next pandemic."

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