The race to develop a COVID-19 vaccine

As *MedNous* went to press, data from the World Health Organization (WHO) showed that more than 100 potential vaccines against SARS-CoV-2, the virus responsible for COVID-19, are currently under development.

So far, however, only a handful of vaccine candidates have entered the clinic, although data is starting to emerge on at least one. On 18 May, Moderna Inc, which is developing vaccines based on messenger RNA (mRNA) technology, announced that the first eight subjects taking part in a Phase 1 study of the company's SARS-CoV-2 vaccine mRNA-1273 had seroconverted with neutralising antibody titres reaching or exceeding those in convalescent sera.

The speed with which these new vaccine candidates are being progressed is unprecedented. Two factors are cited as the reason for this rapid progress. The first is the publication by Chinese researchers in January 2020, more than a month before the pandemic was officially declared, of the sequence of the viral genome. The second is that a considerable amount of work had already been done to develop vaccines for earlier coronavirus epidemics, such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), much of it applicable to a SARS-CoV-2 vaccine.

A breakdown of the strategies being employed to develop a SARS-CoV-2 vaccine is shown in Figure 1.

This article analyses the most promising approaches to developing a vaccine, and looks at some of the individual candidates under development.

Subunit vaccines

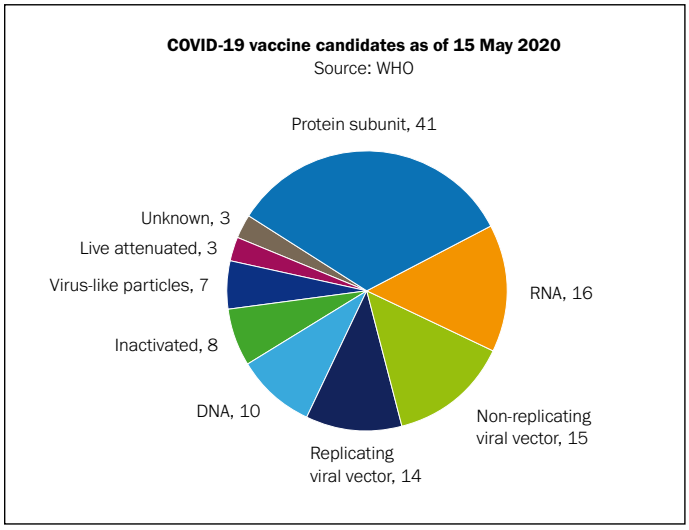
Protein subunit vaccines for SARS-CoV-2 are attracting a lot of attention. They are based on eliciting an immune response against the virus's spike protein (S-protein), thus preventing it from binding to the host ACE2 receptor. This approach has previously been used successfully for other coronavirus infections, such as the 2002-3 SARS outbreak. On that occasion, successful products included vaccines targeting the full-length S-protein as well as the S1 subunit that contains the receptor-binding domain.

None of the SARS-CoV-2 protein subunit vaccines currently being developed has reached the stage of clinical trials, although one, NVX-CoV2373, which is being developed by the US company Novavax Inc, was scheduled to start a Phase 1 trial at two sites in Australia in mid-May. NVX-CoV2373 consists of a full-length recombinant SARS-CoV-2 glycoprotein produced using Novavax's proprietary nanoparticle technology, and incorporates the company's saponin-based Matrix-M adjuvant.

Preliminary results from the trial should be available in July. The vaccine was highly immunogenic in animal models measuring spike protein-specific antibodies, with the already high microneutralisation titres seen after one dose increasing eightfold with a second dose, evidence that the vaccine is likely to be protective in humans. Manufacture of the vaccine has been initiated at specialty biopharmaceutical company Emergent BioSolutions Inc, which says it has the ability to leverage capacity for large-scale manufacturing.

Other companies are also turning to adjuvant technology. Heat Biologics Inc is collaborating with the University of Miami Miller School of Medicine to develop a vaccine based on the company's proprietary gp96 technology. Heat shock protein gp96 is a chaperone protein that occurs in all human cells: it is a potent immune adjuvant that has proven effective in over 300 patients in the context of other vaccines.

Elsewhere, Dynavax Technologies Corp is making its toll-like receptor 9 agonist adjuvant, CpG 1018, available for the development of vaccines against COVID-19. CpG 1018 is the adjuvant used in Heplisav-B, the company's recombinant hepatitis B vaccine which is US Food and Drug Administration-approved for use in the US. Dynavax is working with several other organisations to develop vaccines to prevent COVID-19, including GlaxoSmithKline Plc, Clover Biopharmaceuticals, Sinovac Biotech Ltd, Valneva SE and the University of Queensland, Australia.



Meanwhile the Canadian company IMV Inc has used immunoinformatics to predict and identify several hundred epitopes, of which 23 have been selected for their potential to generate neutralising antibodies against SARS-CoV-2. The company is combining one of these epitopes with its DPX lipid-based delivery technology, which enhances the immune response, to create a vaccine, DPX-COVID-19.

In situations like the current one where speed is of the essence, collaboration between various groups is often crucial. One of the most significant collaborations in the subunit SARS-CoV-2 vaccine area is that between two of the leading players in the vaccine market, Sanofi SA and GlaxoSmithKline Plc. Sanofi is contributing its S-protein COVID-19 antigen, which is based on recombinant DNA technology, while GSK will contribute its pandemic adjuvant technology. The aim is to initiate Phase 1 trials in the second half of 2020 and, subject to a satisfactory outcome and regulatory approvals, to complete development by the second half of 2021.

Technology also has an important role to play. For example, EpiVax Inc is using advanced computational tools to accelerate a COVID-19 vaccine candidate ,EPV-CoV19,

for healthcare workers. The vaccine reportedly drives a cell-mediated immune response but does not induce antibodies. EpiVax is also pursuing other avenues of vaccine research, including a pan-coronavirus DNA vaccine being developed in collaboration with Entos Pharmaceuticals Inc.

Nucleic acid vaccines

Nucleic acid vaccines work by introducing a DNA or RNA sequence coding for a specific disease antigen into the body. Once the sequence is translated into the corresponding peptide, an immune response is induced. Nucleic acid vaccines generally elicit both humoral and cellular immune responses, unlike conventional vaccines that elicit only an antibody response. DNA vaccines comprise a plasmid containing the required DNA sequence, which must be incorporated into the host cell DNA in order to direct the production of antigenic proteins. RNA vaccines comprise a naked mRNA strand which can be used directly by the cell's protein manufacturing machinery in the ribosomes.

Apart from producing a broader immune response, RNA and DNA vaccines offer a number of other benefits. Manufacture of DNA vaccines is much easier than with conventional vaccines, as plasmid DNA is simply produced in bacterial culture *in vitro*. The process is easily adapted to different DNA species. mRNA is even more straightforward to manufacture as it is produced enzymatically from a DNA template *in vitro*. As RNA vaccines do not need to be incorporated into the host nucleus to be effective, they are simpler and possibly safer than DNA vaccines. On the other hand, molecule for molecule, DNA vaccines tend to lead to greater protein production than RNA vaccines. It should be borne in mind, however, that no RNA or DNA vaccine has yet been approved for human use, and regulators therefore have limited experience in this area.

In terms of development, the most advanced DNA-based vaccine for SARS-CoV-2 is currently Inovio Pharmaceuticals Inc's INO-4800, which began a Phase 1 trial in 40 patients in the US on 6 April, scheduled to end by November 2020. The vaccine is delivered intradermally using the company's CELLECTRA electroporation device. In addition, Inovio is collaborating with Beijing Advaccine Biotechnology Co to carry out Phase 1 trials in China, and has recently announced that the Coalition for Epidemic Preparedness Innovations (CEPI) has granted it $6.9 million funding to work with the International Vaccine Institute (IVI) and the Korea National Institute of Health to carry out Phase 1/2 trials in South Korea.

Another DNA vaccine project employing electroporation is the Opencorona project based at the Karolinska Institute in Stockholm, Sweden. The project consortium has generated several chimeric SARS-CoV-2 genes and is in the process of selecting the most promising vaccine candidates in animal models before proceeding to Phase 1 trials. The project is supported by €3 million in funding from the EU.

A different type of needleless delivery system is being used by the privately held biotech Immunomic Therapeutics Inc (ITI): the company is developing a DNA vaccine that will exploit the company's UNITE (Universal Intracellular Targeted Expression) technology. ITI is collaborating with EpiVax Inc and PharmaJet Inc on the development, bringing together the UNITE technology with EpiVax's *in silico* epitope prediction tool and PharmaJet's Tropis needle-free Injection System.

Neither the Opencorona vaccine nor the ITI vaccine has yet entered clinical trials. Other companies with potential SARS-CoV-2 DNA vaccines in preclinical development include the Japanese firm AnGes Inc, India's Zydus Cadila, the Thai-French company BioNet Asia, and the Italian firms Takis Srl and EvviVax.

Among the RNA vaccines in development, two have so far entered clinical trials: BioNTech SE's BNT162 and Moderna Inc's mRNA-1273. BioNTech, based in Mainz, Germany, is collaborating with Pfizer Inc and Shanghai, China-based Fosun Pharmaceutical on the development and commercialisation of BNT162, which exploits the company's Lipid-Nano-Particulate mRNA vaccine platform. This technology has demonstrated effectiveness in several other infectious disease models.

The first cohort of BioNTech's Phase 1/2 clinical trial was dosed in Germany, starting on 23 April. And on 5 May, the two companies began a Phase 1/2 trial in the US. The study is designed to determine the safety, immunogenicity and optimal dose level of four mRNA vaccine candidates evaluated in a single, continuous study. Separately, BioNTech is planning trials in China in partnership with Fosun.

Moderna's mRNA-1273, which was mentioned earlier, has recently been granted Fast Track status by the FDA. It encodes for a prefusion stabilised form of the SARS-CoV-2 spike protein, selected by Moderna in collaboration with the Vaccine Research Center at the US National Institute of Allergy and Infectious Diseases (NIAID). A Phase 2 dose-ranging study is expected to begin shortly, and Moderna is finalising the protocol for a Phase 3 study, expected to begin in July. The company has been awarded up to $483 million from the US Biomedical Advanced Research and Development Authority (BARDA) to accelerate development of mRNA-1273.

Like Moderna's other vaccine candidates, mRNA-1273 comprises lipid nanoparticle (LNP)-encapsulated mRNA. Another LNP-encapsulated RNA vaccine against COVID-19 is being developed in Japan by Daiichi-Sankyo, which is working in collaboration with the Japan Agency for Medical Research and Development and the University of Tokyo. The technology has previously been developed in connection with MERS. Meanwhile in China, a joint research team from Fudan University, Shanghai JiaoTong University, and RNACure Biopharma is also currently working on an RNA vaccine that employs LNP technology.

Other groups developing RNA vaccines for SARS-CoV-2 include the German company CureVac AG which, with EU financial support, is planning to begin Phase 1 studies this summer; the State Research Center of Virology and Biotechnology VECTOR, located in Novosibirsk, Russia; and several academic groups.

Viral vectors

Nearly 30 of the vaccines in development, including two that have already entered clinical trials, use a viral vector to deliver genetic material. Viral vectors essentially take advantage of the natural ability of viruses to infect a certain cell type and deliver its genes into the cell nucleus. To create

a vaccine, genetic material from the pathogen in question (in this case, SARS-CoV-2) is piggybacked onto the vector virus's own genetic material.

Some vectors used in vaccines are capable of replication in the body, rather like live, attenuated viral vaccines that can reproduce in the host to confer extended immunity. Examples of replication-capable vectors that are currently being used in experimental SARS-CoV-2 vaccines include measles virus, influenza virus, yellow fever virus and vesicular stomatitis virus (VSV). Other vaccines employ a non-replicating vector, which can be compared to traditional killed vaccines, which do not reproduce. Example of non-replicating vectors that are being studied in the context of SARS-CoV-2 include various adenoviruses, modified vaccinia virus Ankara (MVA), rabies virus and parainfluenza virus.

The most used viral vector, both for SARS-CoV-2 and other types of vaccine, are adenoviruses. While replication-capable forms of adenovirus have been used in some situations, all the adenovirus-vectored SARS-CoV-2 vaccines in development are based on versions that are rendered incapable of replication. This is achieved by deletion of the E1 region of the viral genome, which is essential for replication. Many adenovirus vectors also have the E3 region deleted, which creates more space for the insertion of foreign genes.

The Oxford Vaccine Group

According to WHO data, there are nine SARS-CoV-2 vaccines under development that employ an adenovirus vector, including the two that are in clinical trials. Most attention to date has focused on ChAdOx1 nCoV-19, which is being developed by the Jenner Institute and the Oxford Vaccine Group in the UK. At the end of April, an agreement was announced between the university and AstraZeneca Plc under which the Anglo-Swedish company would be responsible for the global development, manufacture and distribution of ChAdOx1 nCoV-19. The deal also involves Vaccitech Ltd, a spin-out from the University of Oxford, which shares the rights to the platform technology used in the vaccine.

ChAdOx1 nCoV-19 is based on a recombinant chimpanzee adenovirus vector (ChAdOx1) and the genetic material coding for the SARS-CoV-2 spike protein. The Oxford group has previously worked on a number of similar vaccines, including those for influenza, Zika virus and the MERS coronavirus, which Sarah Gilbert, who leads the Jenner Institute's influenza vaccine and emerging pathogens programme, says provided a helpful template for the development of the SARS-CoV-2 vaccine. The first two patients received ChAdOx1 nCoV-19 on 23 April: the aim is to recruit just over 1,000 volunteers who will receive either ChAdOx1 nCoV-19 or a meningococcal vaccine control (MenACWY). Press reports have suggested that if the trials are successful, a vaccine could be available for widespread use later this year.

The other adenovirus-based vaccine to have entered clinical trials is Ad5-nCoV, which is being developed jointly by CanSino Biologics Inc and the Beijing Institute of Biotechnology, China. It is a genetically engineered vaccine that is based on the replication-defective adenovirus type 5 and expresses SARS-CoV-2 spike protein. According to the Chinese Clinical Trial Registry, recruitment of approximately 100 subjects for a Phase 1 study is underway, and a Phase 2 trial is planned, although recruitment for that trial has not yet commenced. The Phase 1 trial will involve up to 500 subjects in three institutions in Wuhan.

A number of SARS-CoV-2 vaccines based on an adenovirus vector are currently undergoing preclinical evaluation. One of the biggest investments in this area is at Johnson & Johnson's Janssen division, which is building a vaccine around an engineered version of adenovirus 26 (Ad26). Separately, Janssen is also testing the Ad26 platform in vaccines against Ebola, HIV, respiratory syncytial virus and Zika virus, and has been awarded $456 million by BARDA to move a SARS-CoV-2 vaccine through clinical trials – funding which J&J has said it will match. Phase 1 studies are scheduled for September, and the company claims to have a 2,000-litre fermentation vessel available which could produce as many as 300 million doses annually.

Elsewhere, the Italian company ReiThera Srl is expected to begin clinical trials of its SARS-CoV-2 vaccine during the middle of 2020. The vaccine is based on a novel simian adenovirus vector that is proprietary to ReiThera.

Given that an effective SARS-CoV-2 vaccine is likely to be needed in developing countries with relatively poor infrastructure, some companies are taking advantage of the fact that adenovirus vectors lend themselves to the development of vaccines that are easy to store or can be administered without the need for injection. The UK firm Stabiltech Biopharma Ltd's OraPro-COVID-19 vaccine, for example, which is scheduled to begin clinical trials in June, is designed to be freeze-dried. It could then simply be mailed to patients, who could orally self-administer. Similarly, US-based Vaxart Inc is using its VAAST oral vaccine platform to develop a SARS-CoV-2 vaccine that could be administered as a tablet. A slightly different approach is being taken by Altimmune Inc, which is collaborating with the University of Alabama, US to develop a single-dose intranasal vaccine.

Interestingly, there appears to be little commercial interest in the use of replication-capable viral vectors. One exception is Tonix Pharmaceuticals, which is collaborating with the non-profit Southern Research to develop TNX-1800, based on Tonix's live modified horsepox virus vaccine platform. The vaccine expresses an unspecified protein from the SARS-CoV-2. Another is Zydus Cadila, which in addition to its DNA vaccine candidate, is developing a live attenuated recombinant measles virus vectored vaccine against SARS-CoV-2. The Indian company is also investigating the use of its pegylated interferon alpha-2b product PegiHep as a possible treatment for COVID-19.

Despite the progress made to date, a number of issues remain. Safety is a major consideration: of particular concern is the risk that a vaccine may exacerbate the very disease it is trying to control. There is also the question of how long the protection afforded by a vaccine will last. Finally, there is the likelihood that no one single vaccine will meet all the needs associated with the pandemic, and that several will be needed.

This article was written by Peter Charlish, PhD, a contributing editor to *MedNous*, and former editor at Informa Plc.