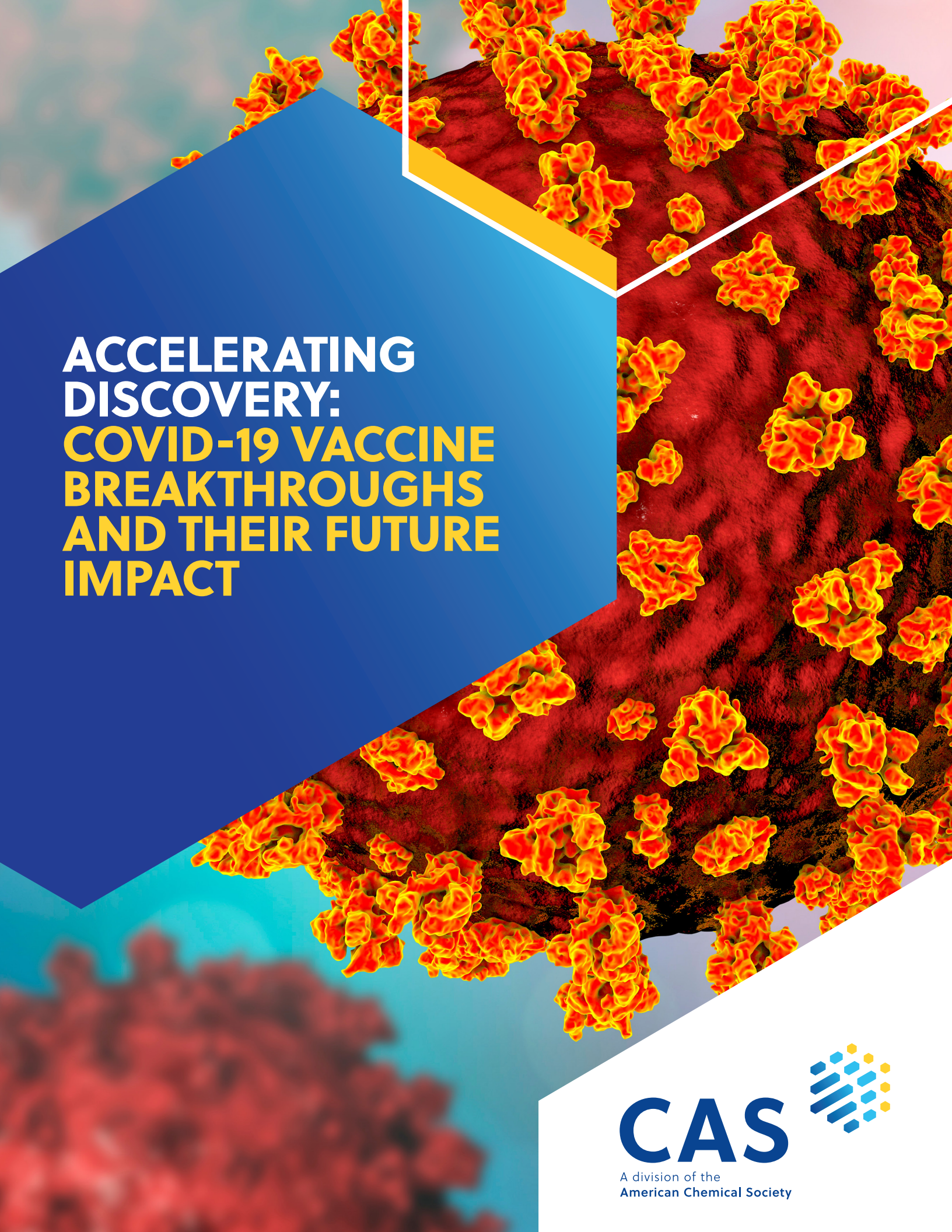

ACCELERATING DISCOVERY: COVID-19 VACCINE BREAKTHROUGHS AND THEIR FUTURE IMPACT

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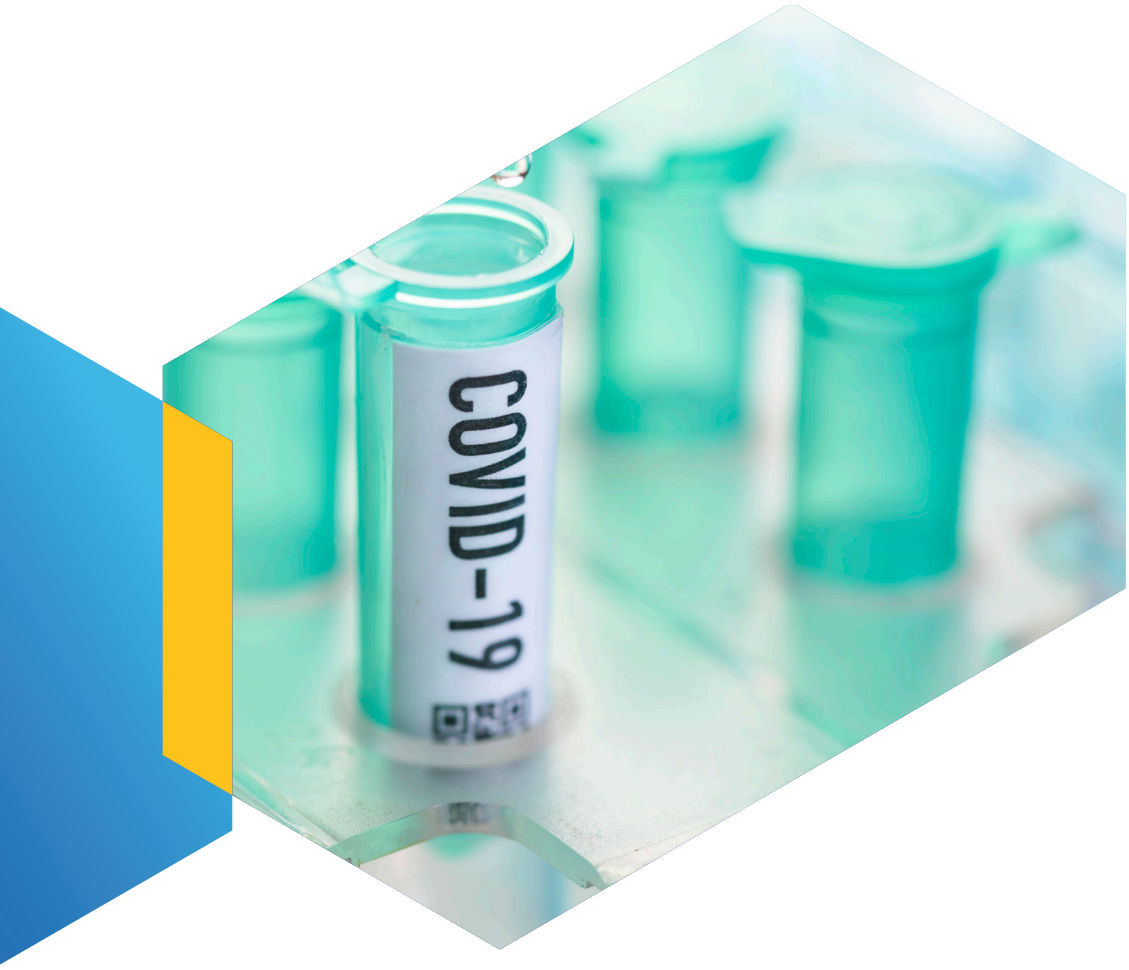


The devastating impact of COVID-19 has driven vaccine development on a scale and speed never seen before, culminating in vaccines receiving conditional approval for use less than a year after the outbreak took hold. To achieve this, researchers have drawn heavily on learnings from earlier vaccine research, particularly those relating to other coronavirus diseases, such as SARS and MERS. In addition, a raft of innovative technologies has been optimized and employed.

As the race to develop COVID-19 vaccines progressed, a variety of potential vaccine platforms were explored, from conventional whole virus vaccines to pioneering mRNA vaccines. During this period of intense research, mRNA vaccines have emerged as a fast, safe and efficient new platform, and ultimately led the race to mass production and approval.

Here we explore some of the innovative technologies and research insights that have played a crucial role in COVID-19 vaccine development and look ahead to exciting future applications.

A period of unprecedented vaccine research

It is hard to overstate the amount of scientific progress made since the first COVID-19 case was detected in late 2019, especially considering that traditional vaccine development often takes 15 years or more. At least 30 countries and regions have been working on COVID-19 vaccines, and over half of these have successfully gotten at least one into clinical trials. **Some vaccine candidates reached clinical trials in less than six months and were conditionally approved within ten – a record speed.**

The first COVID-19 vaccine candidate reached clinical trials in less than six months and was conditionally approved within ten – a record speed.

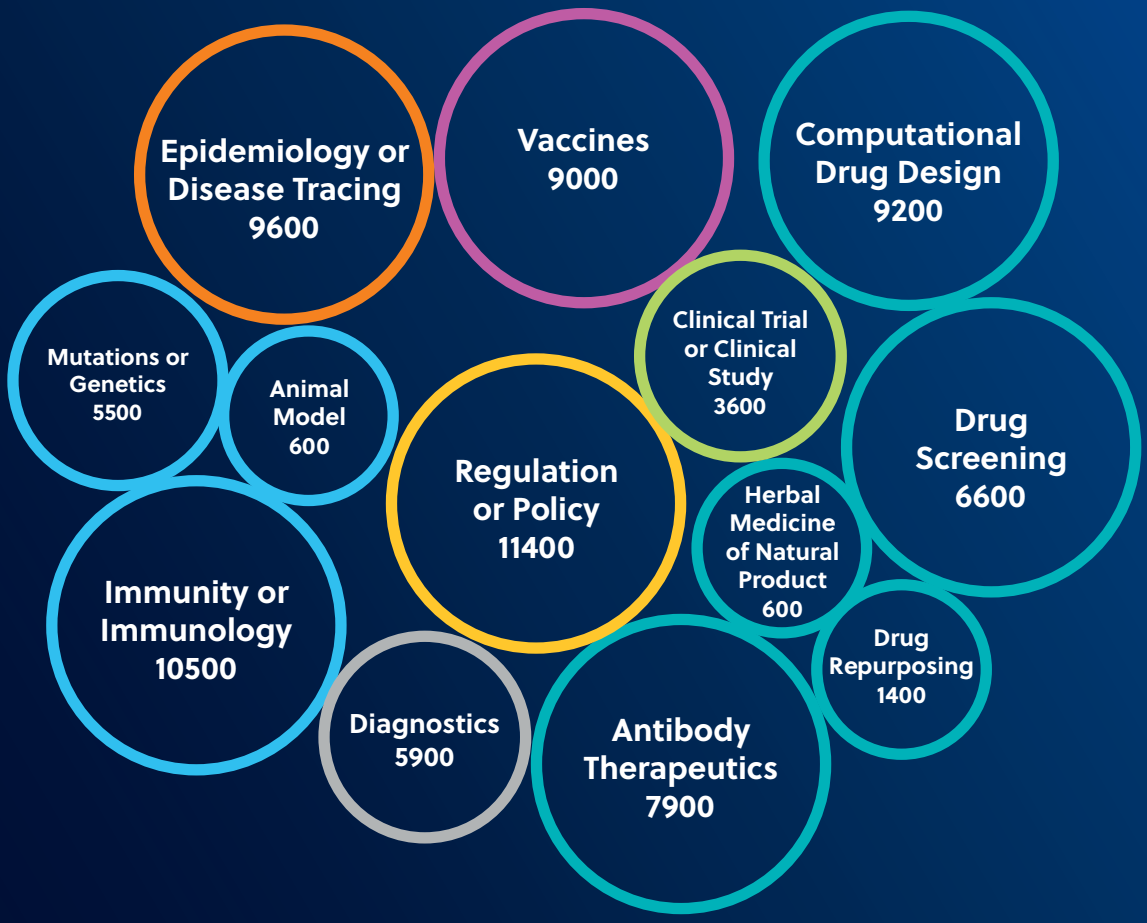


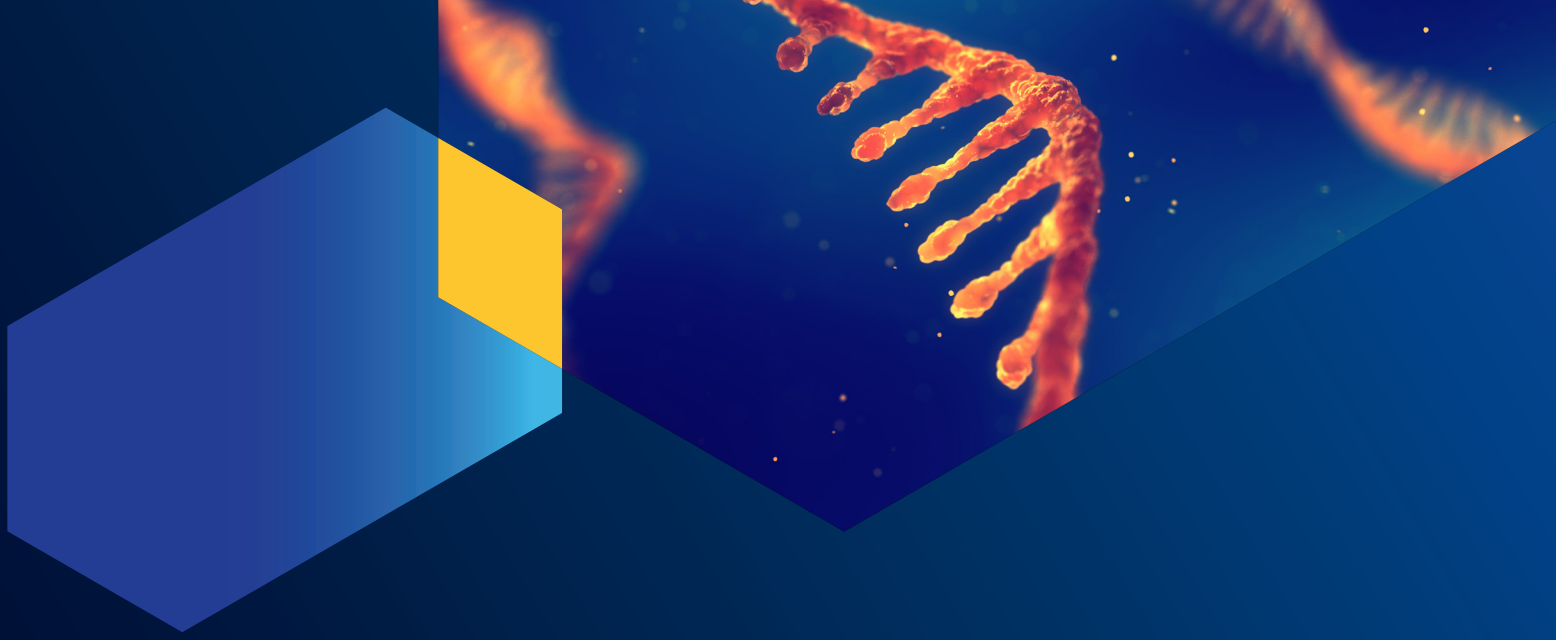
Figure 1. Number of COVID-19 related publications with corresponding key concepts in drug discovery, diagnosis, vaccine development, basic research on SARS-CoV-2, clinical study, epidemiology and regulation & policy. Numbers are rounded to the nearest hundred.

Today, a little over a year since the outbreak began, there are more than 200 candidate vaccines for COVID-19, with a quarter of these already in clinical trials. A few vaccines have also now been given conditional approval by the FDA and the EMA.

To demonstrate the scale and scope of COVID-19 related research, number of documents published as patents or scientific journal articles by the end of 2020 was analyzed according to the key concepts mentioned in the title/abstract area. Figure 1 shows that more than 9,000 patents and scientific journal articles have been published by the end of 2020 that mentioned COVID-19 vaccines in their titles or abstracts. This ranks highly among the wider context of other COVID-19 related concepts in the published documents.

Driven by the highly transmissible nature of the disease combined with the rapidly growing death toll, numerous factors enabled the unprecedented achievement of developing these vaccines in under a year. Based on lessons learned from the 2003 SARS epidemic, international networks and forums now exist to bring together experts from around the world, facilitating information sharing and rapid action to focus R&D effort and maximize impact.1 Such international collaboration between research entities together with support from governments has been key to achieving accelerated research and vaccine development – within four weeks of the World Health Organization (WHO) being alerted to the presence of COVID-19, the SARS-CoV-2 virus had been identified, its genome sequenced and published, RT-PCR diagnostic tests developed, and one of the mRNA candidate vaccines was ready for initial laboratory testing.[1]

This degree of scientific progress is remarkable and will continue to impact research and enhance R&D well beyond COVID-19. **Just as the COVID-19 vaccine development has built on prior studies, future vaccines and therapies will build on the key innovations from the COVID-19 pandemic, including mRNA vaccines and lipid nanoparticle (LNP) technology, as well as new research insights, such as the potential cross-protective effects of vaccines against seemingly unrelated diseases.**



Future vaccines and therapies will build on the key innovations from the pandemic, including mRNA vaccines and lipid nanoparticle (LNP) technology.

mRNA vaccines offer a fast, safe and efficient new platform

The first two vaccines approved for use (in multiple countries) are both mRNA vaccines. In contrast to conventional vaccines, which directly introduce antigenic proteins that stimulate an immune response in the host, mRNA vaccines introduce genetic instructions thereby using the protein-making machinery of the host cells to generate immunogens. In turn, this in situ synthesis of foreign immunogens stimulates both antibody production and T-cell activation offering a double strike against the virus.

Previous research with SARS and MERS suggested that the viral surface Spike (S) protein could be used as the immunogen for COVID-19 vaccine development. So in many COVID-19 mRNA candidate vaccines, the genetic code of the full-length S protein is delivered and translated. This is done within the cytosol of the cell, making mRNA vaccines more efficient than DNA vaccines where the nucleic acid must be delivered into the nucleus instead.

mRNA vaccines are particularly well suited to tackling a highly infectious emerging virus such as SARS-CoV-2. There is increased specificity and efficacy arising from induction of both B- and T-cell immune responses. In addition, mRNA can be produced in large quantities in a cell-free environment by in vitro transcription (IVT) enabling faster development, a simplified production process, and cost-effective manufacturing. When compared with other vaccine approaches, mRNA vaccines offer a highly scalable manufacturing process, which is important for meeting high levels of demand.

mRNA vaccines also confer several safety benefits over other vaccines. Importantly, they do not modify or integrate into the host genome, and there is no presence of virus with mRNA vaccines, meaning they cannot induce the disease they are designed to prevent.

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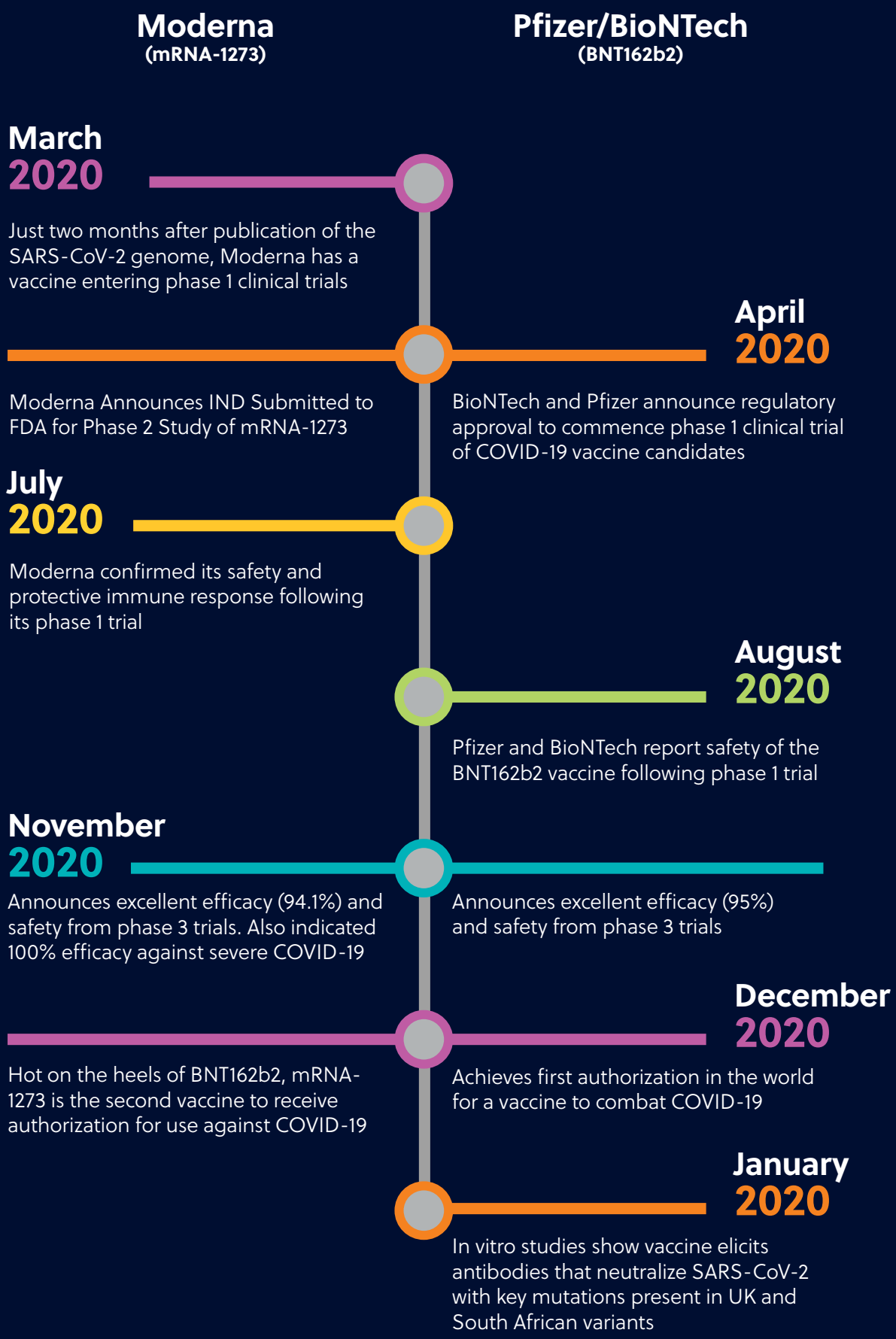
Developing mRNA vaccines for COVID-19 – and beyond

So far, two COVID-19 mRNA vaccines are conditionally approved and there are four more in clinical trials. All are intramuscular vaccines requiring two doses.

Developer/Manufacturer	Vaccine (CAS Registry Number®)	Clinical Stage
Moderna/NIAID (USA)	mRNA-1273 (2457298-05-2)	Phase 3 (EUA approval)
BioNTech (Germany)/Fosun Pharma (China)/Pfizer (USA)	BNT162b1 (2417899-75-1), BNT162b2 (2417899-77-3)	Phase 3 (EUA approval)
Curevac (Germany)	CVNCOV (2541470-90-8)	Phase 3
Arcturus (USA)/Duke-NUS (Singapore)	ARCT-021 (2541451-24-3)	Phase 2
Imperial College London (UK)	LNP-nCoVsaRNA (2545641-90-3)	Phase 1
People's Liberation Army (PLA) Academy of Military	ARCOV (2543878-98-2)	Phase 1

Table 1. mRNA candidate vaccines against COVID-19 currently in clinical trials according to the WHO COVID-19 vaccine landscape.


Moderna
(mRNA-1273)
Pfizer/BioNTech
(BNT162b2)
March
2020
Just two months after publication of the SARS-CoV-2 genome, Moderna has a vaccine entering phase 1 clinical trials
April
2020
Moderna Announces IND Submitted to FDA for Phase 2 Study of mRNA-1273
BioNTech and Pfizer announce regulatory approval to commence phase 1 clinical trial of COVID-19 vaccine candidates
July
2020
Moderna confirmed its safety and protective immune response following its phase 1 trial
August
2020
Pfizer and BioNTech report safety of the BNT162b2 vaccine following phase 1 trial
November
2020
Announces excellent efficacy (94.1%) and safety from phase 3 trials. Also indicated 100% efficacy against severe COVID-19
Announces excellent efficacy (95%) and safety from phase 3 trials
December
2020
Hot on the heels of BNT162b2, mRNA-1273 is the second vaccine to receive authorization for use against COVID-19
Achieves first authorization in the world for a vaccine to combat COVID-19
January
2020
In vitro studies show vaccine elicits antibodies that neutralize SARS-CoV-2 with key mutations present in UK and South African variants

These COVID-19 mRNA vaccines build on three decades of research that has helped to overcome a series of fundamental hurdles. However, there is still work to do. **Research for these vaccines is still ongoing to improve our understanding of the duration of protection, impact on transmission, effectiveness for children or those with chronic conditions, and long-term safety.**

Additionally, researchers are looking at ways to overcome one of the most significant drawbacks of mRNA vaccines, the relatively unstable nature of mRNA. This means that the mRNA vaccines require cold storage: -80°C and -20°C for the BioNTech/Pfizer and Moderna vaccines, respectively. The need for low-temperature storage of mRNA vaccines makes distribution more difficult and introduces additional challenges to large-scale vaccination programs such as those being implemented for COVID-19. However, promising mRNA vaccines that can be stored, and handled at higher temperatures are in development.

The relatively low cost of mRNA plus its efficacy, versatility, and speed of manufacture, make mRNA vaccines a very attractive platform for rapid, large-scale vaccine production. Previously, mRNA vaccines were tested for a range of infectious diseases such as rabies, influenza, and Zika, but none have progressed past small or early-phase trials, partly as there was not enough market demand to expedite the process. In a further example, there is strong need for a cytomegalovirus (CMV) vaccine that can prevent congenital infection and reduce CMV disease in transplant patients. Until recently, the majority of CMV experimental vaccines in development had focused on T cell antigens alone and had shown limited efficacy, however an mRNA vaccine from Moderna is currently in phase 2 trials. The successful approval of the COVID-19 mRNA vaccines could pave the way for other mRNA vaccines to be more readily developed and approved for a wide range of infectious diseases.

The mRNA vaccines also have broader potential applications. For example, cancer cells are known to make proteins that can be targeted by therapeutic mRNA vaccines. This personalized immunotherapy approach involves mRNA vaccines that are custom-made to match the genetic profile of an individual's cancer. For example, Moderna's mRNA-4157 has the potential to enhance clinical outcomes associated with checkpoint inhibitor therapies and a phase 1 study is underway to test the use of mRNA-4157 alone in patients with resected solid tumors and in combination with Merck's Keytruda in subjects with unresectable solid tumors.

Autoimmune diseases may also be treated via mRNA vaccines by controlling auto-reactive T cells without inducing systemic immune suppression. In multiple sclerosis (MS), for example, an mRNA vaccine strategy has been designed to prevent the immune system mistakenly attacking and destroying the myelin of nerve cells. The vaccine restores the body's tolerance to its own proteins, suppressing the characteristic immune over-reactivity of the disease. In studies using mouse models, systemic delivery of purified mRNA that encodes disease-related self-antigens, which normally act as autoimmune triggers, caused the antigens to be presented on lymphoid dendritic cells without provoking an inflammatory immune response [2,3]. This increased tolerance led to more regulatory T cells to suppress the autoimmune response against such antigens, and promoted the suppression of other T cells that attack proteins in myelin. In this study, this mRNA approach resulted in the development of a less severe disease than would normally occur.[4] More research is needed to see if this would be effective in humans, but the technology shows potential.

In theory, mRNA therapeutics could also be used to produce proteins missing in certain diseases, like using chemically modified Cystic Fibrosis Transmembrane conductance Regulator (CFTR) mRNA as a treatment for cystic fibrosis. The RESTORE-CF trial is assessing an RNA-based therapy, MRT5005, that delivers the CFTR mRNA directly into the lungs via nebulization. Again, further research is needed to make this approach clinically relevant and to optimize delivery.

Research into these COVID-19 mRNA vaccines is still ongoing to improve our understanding of the duration of protection, impact on transmission, effectiveness for children or those with chronic conditions, and long-term safety.

Overcoming immunogenicity in mRNA vaccines

One of the major barriers that prevent mRNA vaccines from getting to clinic, is their intrinsic immunogenicity. mRNA vaccines produced by in vitro transcription (IVT) contain an open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (UTRs), a 5′-cap and a 3′ polyadenylation (poly(A)) tail, as shown in Figure 3. Notably, the process of 5′ capping is crucial not only for the initiation of mRNA translation, but also for reducing immunogenicity, as without a cap, the IVT mRNA would be recognized as foreign RNA by the host immune system.

Unmodified IVT mRNA can stimulate Toll-like receptors (TLRs) and activate the innate immune system.[5] Here, to reduce immunogenicity, modified nucleotides can be introduced to the ORF region. This approach has proved successful, for example, the incorporation of pseudouridine (ψ) or N1-methyl-pseudouridine (m1ψ) has shown to reduce immunogenicity and improve translation.[6-7]

As an alternative approach to the incorporation of nucleotide modifications, optimizing the codons with GC-rich sequences may also reduce immunogenicity.[8] In an erythropoietin production model, without chemical modification, theORF of mRNA was engineered by adapting GC-rich codons used for each amino acid. The result showed GC-enriched mRNA may achieve meaningful biological effects without causing inappropriate immunostimulation.

Advances in overcoming the intrinsic immunogenicity of mRNA vaccines could help realise the full potential of this technology by providing the basis for a broad range of possible applications, including the prevention and treatment of infectious and non-infectious diseases, respectively.

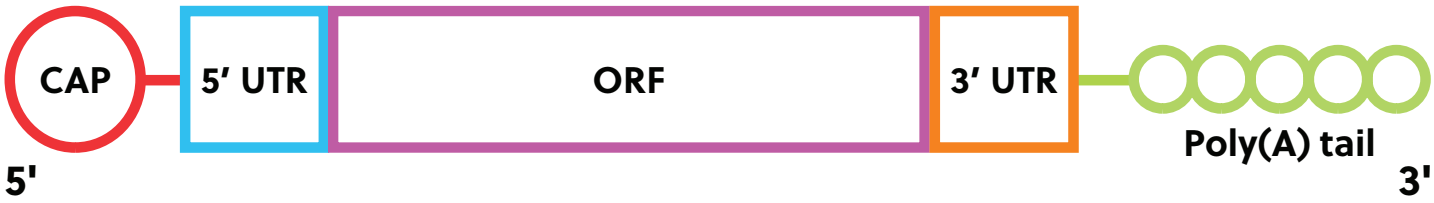


Figure 3: Schematic illustration of an mRNA molecule with the structural elements.

Lipid nanoparticles and the opportunities for innovation in formulations

One of the challenges of using mRNA vaccines is efficient delivery of the mRNA across the cellular plasma membrane to the cytosol. The physicochemical features of nucleic acids, such as their negative charge and hydrophilicity, impede passive diffusion across the plasma membrane. Additionally, a series of barriers, such as association with serum proteins, uptake by the phagocytes, and degradation by endogenous nucleases, obstruct efficient nucleic acids delivery.

To overcome such challenges, LNP technology is increasingly being used to enable the clinical potential of genetic drugs and vaccines. By packaging the mRNA in well-defined nanoparticles, LNPs protect the RNA molecule from in vivo degradation and facilitate intracellular delivery following uptake into target cells by endocytosis. Several mRNA vaccines currently in clinical trials use this LNP technology, as do the COVID-19 vaccines from Moderna and Pfizer-BioNTech. These LNPs have similar compositions, comprising:

- Ionizable cationic lipid – a key component that forms complexes with the anionic nucleic acid
- PEGylated lipid – confers longer systemic circulation of the LNP vehicle by minimizing uptake by macrophages
- Cholesterol – common membrane component
- Phospholipid distearoylphosphatidylcholine (DSPC) as a helper lipid

The two vaccines use different proprietary cationic lipids, but otherwise the ratio of their components is similar. The particle size is in the range 80-100 nm.

Again, the development of LNP technology has been ongoing for some time, with a number of nanoformulations already approved for human use. However, it is the use of LNPs in COVID-19 mRNA vaccines that has brought the technology to the fore, broad potential applications for drug delivery in a wide range of vaccines and therapeutics.

The potential applications for LNPs are many and varied. They have a clear role in genetic medicine where gene editing, vaccine development, immune-oncology and treatment of rare genetic and undruggable diseases all rely on the ability to efficiently deliver nucleic acid into the cell. The RESTORE-CF trial provides evidence of the utility of LNP in mRNA therapy.

LNPs have advantages over other gene and vaccine delivery systems. They are easier to manufacture, are less immunogenic, can carry larger payloads, and can be designed for multiple dosages. As a result, the use and applications of LNPs are likely to increase including ease of manufacture, reduced immune responses, multi-dosing capabilities, larger payloads, and flexibility of design.

The use of LNPs in COVID-19 mRNA vaccines has brought technology to the fore, demonstrating broad potential applications for drug delivery in a wide range of vaccines and therapeutics.

Research insights for future medicine: Do vaccinations provide cross-protection against unrelated viruses?

While the COVID-19 pandemic has driven considerable technological progress, it has also given rise to discoveries about the underlying mechanisms of vaccines and the immunity they provide, which could be of great importance for the future of emerging viruses. For example, researchers have noticed correlations between COVID-19 severity and morbidity and the status of previous, seemingly unrelated, vaccines. For example, it was noted that there was a 28% reduction in COVID-19 risk if people had received a PCV13 pneumonia vaccine in the previous year and a 43% reduction if they had received a polio vaccine.[9] A similar response has also been observed from the Bacillus Calmet-Guerin (BCG) vaccine for tuberculosis.[10]

Further research is needed to understand the molecular mechanisms behind such protection, but it is thought that vaccines could induce metabolic and epigenetic changes that enhance the innate immune response to infections. This process is known as trained innate immunity and may prove to be of considerable importance in tackling any future outbreaks.

During the COVID-19 pandemic, cross-protection by previous vaccines was documented in a considerable number of publications. Analysis of the CAS content collection, for example, identified 190 documents that discuss a possible epidemiological correlation between BCG vaccination and COVID-19 severity and morbidity.

- More than half of the documents suggested that routine BCG immunization programs correlate with lower COVID-19 mortality rates,however 34 documents observed no such correlation.
- Influenza and pneumococcal vaccines were also suggested to reduce the severity of COVID-19, with influenza vaccination potentially acting as a non-specific immune stimulator in patients with COVID-19 leading to early activation of the immune system. Again, more than half of the documents discussed a positive effect.
- Countries with recent MMR vaccination programs were noted to have fewer deaths. A degree of sequence homology between the glycoproteins of SARS-CoV-2, measles and rubella may have led to reduced COVID-19 spread and severity.

While it is clear that more studies are needed, the accumulated **evidence suggests that vaccines can have non-specific effects on unrelated infections and diseases and thus offer some degree of cross-protection. In addition to offering additional potential routes to reduce the spread of future diseases, this could also buy time for targeted vaccines or effective therapies to be developed or identified.**

> **Vaccines can have non-specific effects on unrelated infections and diseases, offering cross-protection. This new understanding is just one of many research findings that have come from the intense focus on COVID-19 and which carries broader implications for future medicine.**

Strengthened foundations for future development

Building on extensive prior research in vaccine development, the scientific community devoted massive efforts to COVID-19 vaccine research. As a result, many innovative approaches to producing vaccines for COVID-19 were adopted and our understanding of vaccines and biology has been substantially increased, which will heavily influence future research.

Basic research has provided a greater understanding of vaccine mechanisms and the cross-protection they provide against a range of diseases. In addition, lessons gained from the successful development of mRNA vaccines as well as the use of LNP delivery systems will accelerate the development of vaccines and genetic therapies for a variety of diseases and medical conditions, delivering long-term benefits for science and humanity.

The COVID-19 crisis has enabled scientists to achieve unprecedented progress. However, it will only be over the coming years that we will begin to appreciate the full potential of the technologies and insights yielded from the development of innovative new vaccines to COVID-19 and start to fully realize their promise for future medicine.

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