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Vaccine manufacturing facilities have had to rapidly ramp up their capabilities to produce RNA vaccines.

HOW COVID UNLOCKED THE POWER OF RNA

Vaccine research and development might never be the same again. **By Elie Dolgin**

It was a Friday afternoon in March 2013 when Andy Geall got the call. Three people in China had just become infected with a new strain of avian influenza. The global head of vaccines research at Novartis, Rino Rappuoli, wanted to know whether Geall and his colleagues were ready to put their new vaccine technology to the test.

A year earlier, Geall's team at Novartis's US research hub in Cambridge, Massachusetts, had packaged strings of RNA nucleotides inside of small fat droplets, known as lipid nanoparticles (LNPs), and used them to successfully vaccinate rats against a respiratory virus[1]. Could they now do the same for the novel flu strain? And could they do it as fast as possible?

As Geall, head of the RNA group, recalls: "I said, 'Yeah, sure. Just send us the sequence.'" By Monday, the team had begun synthesizing the RNA. By Wednesday, they were assembling the vaccine. By the weekend, they were testing it in cells – a week later, in mice[2].

The development happened at a breakneck speed[3]. The Novartis team had achievedin one month what typically took a year or more.

But at the time, the ability to manufacture clinical-grade RNA was limited. Geall and his colleagues would never find out whether this vaccine, and several others that they developed, would work in people. In 2015, Novartis sold its vaccines business.

Five years and one global pandemic later, RNA vaccines are proving their worth. Last month, two RNA vaccine candidates – one from US pharmaceutical giant Pfizer and BioNTech in Mainz, Germany, and another from Moderna in Cambridge, Massachusetts – won emergency approval from regulators in several countries to fight COVID-19.

The era of RNA vaccines has arrived – and dozens of companies are getting in the game. "All of the major pharmas are, in one way or the other, now testing out the technology," says Jeffrey Ulmer, former head of preclinical research and development at GlaxoSmithKline's vaccine division in Rockville, Maryland, and before that a member of Geall's team at Novartis.

The idea of using RNA in vaccines has been around for nearly three decades. More streamlined than conventional approaches, the genetic technology allows researchers to fast-track many stages of vaccine research and development. The intense interest now could lead to solutions for particularly recalcitrant diseases, such as tuberculosis, HIV and malaria. And the speed at which they can be made could improve seasonal-flu vaccines.

But future applications of the technology will run up against some challenges. The raw materials are expensive. Side effects can be troubling. And distribution currently requires a costly cold chain — the Pfizer–BioNTech COVID-19 vaccine, for example, must be stored at −70 °C. The urgency of COVID-19 is likely to speed up progress on some of those problems, but many companies might abandon the strategy once the current crisis subsides. The question remains: where will it end up?

"The RNA technology has proved itself, but it's not done yet," says Philip Dormitzer, head of viral vaccines research at Pfizer, and a former colleague of Geall's at Novartis. "And now that we've seen it work for COVID-19, it's tempting to want to do more."

Small particles, big advance

Vaccines teach the body to recognize and destroy disease-causing agents. Typically, weakened pathogens or fragments of the proteins or sugars on their surfaces, known as antigens, are injected to train the immune system to recognize an invader. But RNA vaccines carry only the directions for producing these invaders' proteins. The aim is that they can slip into a person's cells and get them to produce the antigens, essentially turning the body into its own inoculation factory.

The idea for RNA-based vaccination dates back to the 1990s, when researchers in France (at what is now the drug firm Sanofi Pasteur) first used RNA encoding an influenza antigen in mice[4]. It produced a response, but the lipid delivery system that the team used proved too toxic to use in people. It would take another decade before companies looking at RNA-interference therapeutics — which rely on RNA's ability to selectively block the production of specific proteins — discovered the LNP technologies that would make today's COVID-19 vaccines possible.

"Finally, there was the breakthrough," says Nick Jackson, head of programmes and innovative technologies at the Coalition for Epidemic Preparedness Innovations in Oslo, a global partnership to accelerate vaccine development. "That was really the watershed that allowed the application of messenger RNA to a whole range of different disease indications."

In 2012, around the time that Geall and his colleagues described[1] the first LNP-encapsulated RNA vaccine, the US Defense Advanced Research Projects Agency (DARPA) began funding groups at Novartis, Pfizer, AstraZeneca, Sanofi Pasteur and elsewhere to work on RNA-encoded vaccines and therapeutics. None of the big-name firms stuck with the technology, however. "They were reticent about taking on any risk with a new regulatory pathway for vaccines, even though the data looked good," says Dan Wattendorf, a former programme manager at DARPA.

But two smaller firms with ties to the DARPA programme continued to work on the technology. One was CureVac in Tübingen, Germany, which began human testing of a rabies vaccine in 2013 (ref. 5). CureVac also has a COVID-19 vaccine in late-stage testing.

The other was Moderna, which built on work funded by DARPA to eventually bring an RNA-based vaccine for a new strain of avian influenza into clinical testing in late 2015. It elicited strong enough immune responses[6] that the company moved ahead with human trials of RNA vaccines for cytomegalovirus (a common cause of birth defects), two mosquito-borne viruses (chikungunya and Zika) and three viral causes of respiratory illness in children.

GlaxoSmithKline, which had acquired most of Novartis's vaccine assets, also began evaluating an RNA-based rabies vaccine in 2019.

That was the full extent of clinical development for RNA vaccines at the beginning of 2020: only a dozen candidates had gone into people; four were swiftly abandoned after initial testing; and only one, for cytomegalovirus, had progressed to a larger, follow-on study.

Then came the coronavirus — and with it, "there's been this enormous spotlight", says Kristie Bloom, a gene-therapy researcher at the University of the Witwatersrand in Johannesburg, South Africa. In the past ten months alone, at least six RNA-based COVID-19 vaccines have entered human testing. Several more are nearing the clinic.

"This technology is still super early, and we're going to see multiple iterations over the coming years."

Need for speed

RNA vaccines seem built for speed. From the genetic sequence of a pathogen, researchers can quickly pull out a potential antigen-encoding segment, insert that sequence in a DNA template and then synthesize the corresponding RNA before packaging the vaccine for delivery into the body.

Moderna, for example, managed this within 4 days of receiving the SARS-CoV-2 genome sequence. It focused on the virus's spike protein, a surface protein used to enter cells. Collaborating with the US National Institutes of Health, the company then ran proof-of-concept experiments in mice before kicking off first-in-human testing in a span of just two months.

Any vaccine, in theory, could be created in the same way. "It truly is a platform in that sense," says John Shiver, head of vaccine research and development at Sanofi Pasteur. With RNA, "you don't have to recreate the entire process".

Classical approaches to vaccine creation, by contrast, require bespoke, expensive and time-consuming steps for every candidate. These inefficiencies help to explain why health authorities must choose which strains to put into each year's seasonal-flu vaccine months ahead of flu season. Those choices often miss the mark, and there is no time to go back and test an alternative. As a result, flu vaccines are rarely more than 60% effective.

With RNA, however, vaccine makers could more quickly pivot to an effective selection of antigens. "You could theoretically move very fast to adjust sequence and address that — almost on the fly," says Ron Renaud, chief executive of Translate Bio, an RNA-focused company in Lexington, Massachusetts, that is working with Sanofi Pasteur on RNA-based vaccines for influenza, COVID-19 and several other viral and bacterial pathogens.

Thanks to their plug-and-play functionality, RNA vaccines could aid basic research. Justin Richner, a vaccinologist at the University of Illinois College of Medicine in Chicago, is developing a RNA-based dengue vaccine in his own laboratory. Richner and his colleagues routinely chop and change the gene sequence encoding the envelope protein that the dengue virus uses to launch its attack on human cells. By iterating their design, the researchers have tested around 15 vaccine candidates in mice.

"It's really easy to manipulate the coding sequence of the vaccine to try new hypotheses and strategies that may make for better vaccines," says Richner.

Other treasures

Advances in the technology are now helping researchers to close in on some of the holy grails of vaccine development — such as a universal flu shot that would work against any strain of the virus without being redesigned each year. Others are eyeing jabs against HIV and other top killers in lower-income countries. Such vaccines have eluded scientists often because of the way that pathogens systematically alter their surface proteins to evade immune recognition. Some infectious agents, such as malaria, also have elaborate life cycles that further complicate the process of picking antigens.

RNA vaccines could include instructions for multiple antigens, either strung together in a single strand, or with several RNAs packaged together in a single nanoparticle.

Norbert Pardi, a vaccine scientist at the University of Pennsylvania Perelman School of Medicine in Philadelphia, took the latter approach for his experimental flu vaccine. Made of four RNA strands, each encoding a different influenza protein, the multiplex vaccine successfully protected mice from infection with one particular subtype of influenza virus[7].

Now, Pardi and his collaborators at the Icahn School of Medicine at Mount Sinai in New York City hope to repeat the exercise for the other 2 main viral subtypes before putting everything

together into a 12-strand flu shot that could supplant the need for annual vaccination. "If you hit the virus at multiple points," Pardi says, "you can induce broadly protective immune responses."

Stability and safety

Despite its many potential advantages, today's RNA-vaccine technology leaves room for improvement. "This technology is still super early," says Robin Shattock, an immunologist at Imperial College London, "and we're going to see multiple generations and iterations over the coming years, I suspect."

First, there's the issue of cold storage. Both the Pfizer–BioNTech and Moderna vaccines require cold temperatures to maintain the integrity of their RNA.

But at least two companies claim to have COVID-19 RNA vaccines that are stable for months at warmer temperatures.

CureVac, which uses the same LNPs as Pfizer–BioNTech, folds its RNA into compact 3D structures, which allows for storage at refrigerated temperatures for months, says chief technology officer Mariola Fotin-Mleczek. And Suzhou Abogen Biosciences, a Chinese company with an RNA vaccine for COVID-19 now in early human testing, has focused on LNP quality and purity to create a product that reportedly maintains its potency for up to one week at room temperature[8].

There's another challenge: so far, RNA vaccines tested for human use against disease, COVID-19 or otherwise, have generally required a double dose to be effective. And judging by poor compliance with other multi-dose vaccines, many people who get the first shot probably won't get the second.

New delivery systems could fix that. At Vaxess Technologies in Cambridge, Massachusetts, for example, researchers have developed a wearable skin patch studded with tiny silk-tipped, dissolvable microneedles that slowly trickle vaccine into the body.

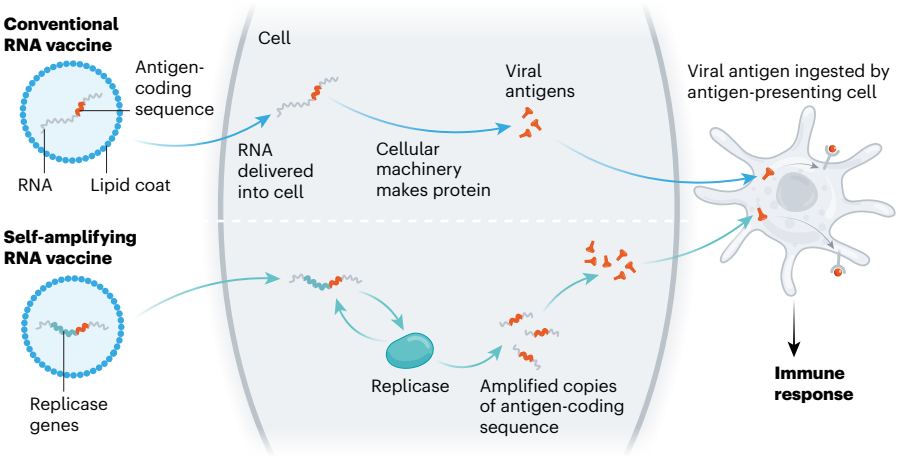
Administering the vaccine in drips instead of all at once could help to solve a third drawback: side effects. Severe reactions, although transient, do seem to be more common with COVID-19 shots than with other immunizations – more than 80% of people who received the Moderna vaccine in clinical trials had some type of systemic reaction to the shot, with bouts of fatigue, muscle pain and other issues that often proved briefly debilitating.

That unpleasantness might be acceptable in the midst of a deadly global pandemic, says vaccinologist Stanley Plotkin, who consults for many vaccine manufacturers. But people might baulk at routinely feeling so ill for, say, their annual flu shot. And for any vaccines geared toward infants, "one would certainly want to have something less reactogenic".

Contaminants in vaccine synthesis and the LNP delivery system are thought to be two of the main sources of reactogenicity. Purification systems are only so good, and LNPs can be optimized only so much. For these reasons, vaccine manufacturers often administer lower doses to limit a person's exposure to both. With a conventional RNA vaccine, lower doses mean lower potency. But companies such as Arcturus Therapeutics in San Diego, California, and VaxEquity in London have devised workarounds by creating self-amplifying RNA constructs for their COVID-19 vaccines (see 'How RNAs can work harder').

HOW RNAS CAN WORK HARDER

RNA vaccines work by tricking the body's cells into producing a fragment of a virus, an antigen, from an RNA template. One strategy to make them more effective at lower doses — or in a single dose — is to incorporate the instructions for assembling a replicase, which can make lots of copies of the RNA template for producing antigens.



In small doses

Unlike the front-runner RNA-based vaccines, which contain little more than the coding sequence for the coronavirus spike protein flanked by regulatory regions on either end, these self-replicating vaccine candidates also include instructions for the RNA to copy itself.

The vaccine constructs are a bit clunkier, requiring more sequence optimization and manufacturing finesse. But they allow companies to lower the dose. And the replicating RNA more closely mimics a natural viral infection – triggering a stronger, broader immune response, which might allow for single-dose inoculation regimens.

BioNTech has improved on the amplifying technology[9]. Before COVID-19, the company focused mostly on cancer vaccines. But with a proven reputation, expanded production capacity and substantial cash flow expected from sales of the COVID-19 vaccine, "that will allow us to expand on the infectious-disease platform much faster", says BioNTech co-founder and chief executive Uğur Şahin.

Ziphius Vaccines in Oostkamp, Belgium, has similarly tried to capitalize on coronavirus momentum. Founded in May 2019 – initially to develop RNA-based treatments for rare diseases such as Duchenne muscular dystrophy and cystic fibrosis – Ziphius overhauled its development plans last year after starting to work on a self-amplifying RNA vaccine for COVID-19. Chief executive Chris Cardon says the start-up is now trying to raise €30 million (US$37 million) to advance 14 preclinical programmes for a variety of infectious diseases.

RNA vaccines might yet face financial headwinds. Many industry insiders don't expect the current white-hot interest to last once the pandemic subsides.

"It's pretty hard to talk people into taking bets on this type of technology for vaccines in infectious diseases," says Nathaniel Wang, chief executive of Replicate Bioscience in San Diego, California, a company he co-founded last year with Geall to develop RNA-based treatments for cancer. And although Replicate has forged some academic and commercial partnerships around RNA vaccines for COVID-19 and Zika, it's not what most venture-capital firms want to fund, says Wang.

Still, with RNA vaccines making headlines, Geall and many of his former colleagues have been replaying their days at Novartis. Had the company not sold off its vaccines unit, could they have helped to stamp out Ebola or Zika outbreaks in the past decade?

"There's always a little bit of sadness looking back," says Christian Mandl, former head of research and early clinical development at Novartis's vaccines unit. But he takes solace in the success of the COVID-19 vaccines today. "I am very proud that we made a valuable contribution."

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