INJECTION OF HOPE

Messenger RNA could help to boost immunity against cancer, influenza and much more.

Brad Kremer received an experimental vaccine for his cancer.

KATYE MARTENS BRIER

BY ELIE DOLGIN

Brad Kremer had waited months to receive an experimental cancer vaccine called BNT122, during which time the melanoma on his skin had spread to his liver and spine. His back pain was getting worse, he was rapidly losing weight and new cancerous lesions kept appearing on his left thigh. "It was very scary," says Kremer, a 52-year-old sales representative from Acton, Massachusetts.

But within weeks of his first injection in March, Kremer could see that the vaccine was working. The coin-sized melanoma spots that popped up from his skin were now flat discolourations measuring millimetres across. "I was actually witnessing the cancer cells shrinking before my eyes," he says. Several doses later, his appetite has returned, his back pain has subsided and scans show that his cancer is continuing to retreat.

Kremer's dramatic response exemplifies the medical potential of vaccines built on messenger RNA. In this method, strings of lab-synthesized nucleotides train the immune system to recognize and destroy disease-causing agents — be they cancer cells or infectious viruses.

Other ways of making vaccines can achieve the same therapeutic objective. But the potency, versatility, speed of manufacturing and low cost of mRNA make it an attractive platform for the rapid development and large-scale production of new or custom-made vaccines.

Early clinical results have demonstrated the technology's promise. Researchers at BioNTech in Mainz, Germany, the manufacturer of the cancer vaccine that Kremer is receiving, reported in 2017 that all of the first 13 people with advanced-stage melanoma to receive the personalized immunotherapy — which is tailor-made to match the genetic profile of each person's cancer — showed elevated immunity against the mutated bits of their tumours. As a result, these patients' risk of developing new metastatic lesions was significantly reduced[1]. For viral diseases, prophylactic vaccine candidates against rabies[2] and pandemic influenza[3] have each proved safe and induced protective antibody responses in healthy volunteers. In both cases, however, the antiviral effects waned after less than a year, suggesting that improvements are needed to provide more robust and long-lasting immunity.

"There's a lot of potential here," says John Mascola, director of the Vaccine Research Center at the US National Institutes of Health in Bethesda, Maryland. "It's still early in the development of these vaccines, but the platform has shown proof of concept."

MADE TO MEASURE

Moderna Therapeutics is racing to develop mRNA vaccines further. In 2018, company executives cut the ribbon on a US$130-million, two-storey, football-pitch-sized manufacturing plant in Norwood, Massachusetts, about one hour's drive from the company's headquarters in Cambridge. Technicians at the new site synthesize and formulate mRNA for all of Moderna's early-stage clinical trials, including for an ongoing investigation of mRNA-4157, a personalized cancer vaccine that, like BioNTech's BNT122, has shown preliminary signs of anti-tumour activity in people with cancer.

Along with other companies testing the same strategy, Moderna starts the process of making its personalized treatment by taking a pair of genetic profiles from each individual: one from a biopsy of the tumour, the other from a vial of healthy blood cells. Algorithms

compare the DNA sequences of the two samples and produce a list of 34 targets, each encoding a different mutant protein expressed by the cancer that is predicted to be useful in training the immune system to attack the disease.

The technicians then take those digital sequences from the computer model and turn them into physical products. First, they codify the cancer-specific mutations into a ring-shaped DNA molecule called a plasmid. They then convert the DNA into strands of mRNA before the therapeutic nucleotides are finally coated in lipid nanoparticles to make them more stable in the body.

Throughout this month-long manufacturing process, each patient's tailor-made vaccine is assigned a colour — red, blue, yellow or green — and technicians wear colour-coded hairnets, work behind colour-matched lines on the laboratory floor and use only colour-coordinated equipment, all as a visual precaution against mix-ups. "We need to make sure it's one batch per patient all the way," explains Jennifer White, head of quality assurance at the site.

At the American Society of Clinical Oncology's annual meeting in June, Moderna reported first-in-human clinical data showing that mRNA-4157 can generate mutation-specific immune responses in people with cancer. And when administered with a checkpoint inhibitor — an antibody drug designed to further ramp up the body's cancer-fighting immune activity — the therapy also shrank tumours in 6 of 20 participants with metastatic disease. Moderna and its development partner, Merck, based in Kenilworth, New Jersey, launched a randomized, 150-person follow-up study in July.

As personalized mRNA vaccines go through trials, Moderna, BioNTech and CureVac, based in Tübingen, Germany, are simultaneously developing off-the-shelf vaccine candidates as well. These ready-made vaccines are not as immunogenic as the most potent customized vaccines for people with highly mutated cancers, but they are potentially suitable for everyone. There is no lengthy customization process; no long waits, often of a month or more; and no added labour and manufacturing costs. They are ready for anyone who needs them.

A NEED FOR SPEED

Oncology is one important area for mRNA vaccine manufacturers, but they are also developing products to tackle infectious diseases. They are taking advantage of the platform's ease of manufacture to create rapid-turnaround products. And they are deploying the technology to combat a handful of viruses that have remained impervious to conventional vaccine strategies.

One such target is cytomegalovirus (CMV), the most common infectious cause of neurological defects in newborns in the developed world. A vaccine is desperately needed to prevent pregnant women from passing the virus to their developing fetuses. But vaccine makers have struggled to recreate the virus' pentameric complex — a bundle of five proteins that mediate entry and exit into human cells — in a way that generates a robust immune response when introduced in the body.

"You can't make five different things in a vat, purify them and then try to put them together in the lab," says Tal Zaks, Moderna's chief medical officer. "They have to be put together within the cell," he says, "and mRNA allows researchers to do that."

Moderna scientists demonstrated this last year, creating a multi-sequence mRNA vaccine that prompted cells transfected with the mRNA to express the full pentamer on their surfaces, eliciting protective antibody responses in immunized mice and monkeys[4]. And last month, the company disclosed that participants in a phase I human trial experienced dose-dependent increases in antibody levels as well. Other prophylactic vaccines in Moderna's pipeline with promising early clinical data include one for respiratory syncytial virus, a common cause of airway inflammation in infants. And in the case of the company's mRNA-1653, a dual vaccine against two other recalcitrant respiratory viruses, metapneumovirus and a type of parainfluenza, which are also responsible for severe lung infections, the success of Moderna's early trials shows that "you can actually now do combinations", Zaks says.

Moderna is also working on vaccines to tackle emerging infectious diseases such as avian influenza and Zika virus, for which the speed of mRNA manufacturing could be beneficial in the event of a pandemic. "One of the greatest advantages of this mRNA strategy is just how fast you can go from a nucleotide sequence to a vaccine product," says Justin Richner, a vaccine researcher at the University of Illinois College of Medicine in Chicago, who has collaborated with Moderna in the past.

The current speed record was set in 2013 in response to an influenza outbreak in China: it took scientists at Novartis just eight days to make a vaccine candidate. Health officials at the Chinese Center for Disease Control and Prevention in Beijing posted gene sequences from the virus on a data-sharing platform on a Sunday in late March[5]. By the following Sunday, Andy Geall and his team at Novartis's vaccine unit in Cambridge, Massachusetts, were already running validation experiments of their mRNA vaccine in hamster kidney cells.

"It happened in real time the moment that sequence was available," says Geall, who is now head of formulations, analytics and chemistry at Avidity Biosciences in La Jolla, California. For a conventional vaccine, the same process could take six months or more, he adds.

"YOU CAN USE MESSENGER RNA VACCINES FOR PRETTY MUCH EVERYTHING."

Geall continues to consult with companies and academics working on mRNA vaccines. He has recently started to go beyond viruses to other pathogens. Last year, for example, he joined forces with clinical immunologist Richard Bucala's team at Yale School of Medicine in New Haven, Connecticut, and successfully used mRNA to vaccinate mice against malaria[6], which is caused by a single-celled parasite.

"The whole platform is very, very flexible," says Norbert Pardi, who studies infectious diseases at the University of Pennsylvania's Perelman School of Medicine in Philadelphia. He is currently working on vaccines for malaria as well as HIV, hepatitis C and several other viral diseases. "You can use mRNA vaccines for pretty much everything," he says.

TAKE ANOTHER SHOT

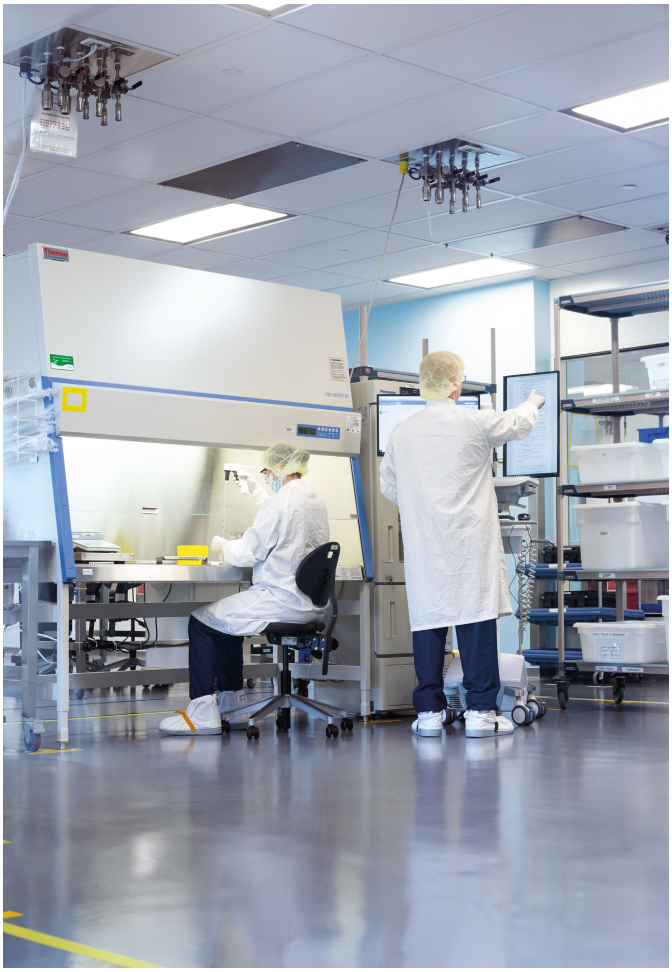
Developing mRNA vaccines is not always straightforward, however. Moderna's initial candidate vaccine for Zika virus, for example, was well tolerated in people but failed to provoke much of an immune response. With funding from the US government, Moderna went back to the lab, optimized the vaccine sequence and developed another candidate that is, according to Zaks, "at least 20 times more potent" than the first-generation product in mice and monkey testing.

The first clinical-stage mRNA vaccine from CureVac was also disappointing. It was a rabies vaccine[2] that could induce antibody responses in some study participants, but only when administered through needle-free devices — and even then, only around two-thirds of vaccinated people achieved the recommended level of antiviral therapy.

CureVac has since altered its delivery platform and restarted human trials with a vaccine candidate that is encased in lipid nanoparticles. As the company's scientists reported in 2017, this change enhances the cellular uptake of the mRNA sequences, so the same level of antibody and T-cell responses can be achieved in mice and monkeys using a tiny fraction of the dose[7].

But starting clinical development again cost the company valuable time in the race to bring its products to market. For chief technology officer Mariola Fotin-Mleczek, this was an important lesson learnt the hard way. "Formulation makes a big impact," she says.

What goes into the mRNA sequence matters a great deal, too. Some companies and non-profit groups are pushing ahead with



MODERNA

Technicians at Moderna make a personalized cancer vaccine.

'self-amplifying' mRNA vaccines. These include everything found in a conventional mRNA vaccine, plus all the genes that encode the RNA replication machinery. This combination of genes allows one strand of synthetic mRNA to generate thousands of copies of the mRNA of interest, says Jeffrey Ulmer, head of preclinical research for vaccines at GlaxoSmithKline in Rockville, Maryland. This amplification effect, he points out, "gives you the potential to have a much more potent immune response with the same dose of RNA — or alternatively, you need much less RNA to get an equivalent level of immune protection".

For instance, BioNTech scientists, working with immunologists from Imperial College London, reported that mice could be fully protected from influenza using just 1.25 micrograms of self-amplifying mRNA — a small fraction of the 80 micrograms of a conventional mRNA vaccine needed to produce the same effect[8]. Late last year, scientists at Imperial collaborated with the Coalition for Epidemic Preparedness Innovations, a global public–private partnership based in Oslo, to advance its self-amplifying mRNA platform to produce vaccines against rabies, the Marburg virus and H1N1 influenza.

Beyond vaccines, mRNA technologies can be used to produce the missing or defective proteins responsible for all manner of diseases. Many companies, including Translate Bio of Lexington, Massachusetts, are pushing ahead with this kind of restorative therapeutic strategy. But as Translate Bio's chief scientific officer, Richard Wooster, points out, targeting corrective mRNA drugs to the desired organs and cell types in the body muscle, say, or to cancer cells, is extremely difficult. In addition, scientists are still struggling to devise chemical formulations of mRNA that avoid unwanted immune reactions to the molecules.

Vaccines are not hampered by these technical complexities. "With vaccination, you're getting a systemic effect, so you don't necessarily need to have specific tissue targeting," says Wooster, whose company signed a deal in 2018 with Sanofi Pasteur of Paris to develop mRNA vaccines for up to five pathogens. Furthermore, because the goal with any vaccine is to elicit immune activity, the tendency for lab-made mRNA to trigger the immune system — and thus serve as its own immunological enhancer — becomes a feature, not a problem.

Most of the mRNA vaccines developed so far have focused on cancer and infectious diseases. But BioNTech also holds the patent rights to an mRNA-vaccination platform designed to protect against allergens such as grass pollen and house dust mites. "I think it would be a very elegant way to prevent allergies," says Richard Weiss, an immunologist at the University of Salzburg, Austria, who helped to develop the technology. His team showed that mRNA-based immunization can completely protect mice from developing allergies against timothy grass, a common cause of hay fever[9].

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All companies have to set strategic priorities, however, and for now BioNTech's remain squarely rooted in oncology. Last month, its scientists reported at a cancer immunotherapy conference in Paris that the company's most advanced off-the-shelf vaccine candidate helped to shrink or stabilize melanoma in 19 of 42 early-trial participants. In continued follow-up investigations from the firm's first-in-humans study of its personalized cancer vaccine, all the people who had previously responded favourably were still relapse-free up to 41 months after treatment. And those responses came without the addition of a checkpoint inhibitor.

BioNTech is banking on the idea that the combination that Kremer is receiving for his metastasized melanoma will yield even better results, especially now that most of the kinks have been ironed out. Back in February, Kremer was supposed to receive his first dose of personalized vaccine shortly before taking a holiday to the Florida Keys with his wife and two teenage daughters. But the treatment, which BioNTech manufactures in Germany and ships to patients around the world, was held up at customs. The dry ice used to chill the vaccine vaporized, so the product thawed out and had to be discarded.

Kremer says he maintained a positive outlook as he waited three weeks for the next shipment of vaccine to arrive. His wife, Ginny, was less patient. "She was very upset," Kremer says. "I don't think she slept for two months."

BioNTech has since developed systems to ensure that its personalized vaccines reach people as quickly as possible — a crucial feature for any cancer treatment when time is of the essence. Fortunately for Kremer, the delay didn't impact his prognosis. "He's had a remarkable response," says cancer immunologist Ryan Sullivan, who is treating Kremer at Massachusetts General Hospital in Boston. And although "there are shipping concerns that can happen and not everyone responds", Sullivan adds, "with cases like Brad's, it's easy to get excited about this type of strategy." ■

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