



The BioNTech–Pfizer vaccine against COVID-19 being prepared to vaccinate a patient in the UK. Credit: PhotoEdit / Alamy Stock Photo

## After COVID-19 successes, researchers push to develop mRNA vaccines for other diseases

mRNA vaccines are now in the limelight as a key tool for tackling COVID-19, but the technology was originally developed for other diseases, such as cancer, that researchers are now hoping to treat.

Mike May

When the broad range of vaccines against COVID-19 were being tested in clinical trials, only a few experts expected the unproven technology of mRNA to be the star. Within 10 months, mRNA vaccines were both the first to be approved and the most effective. Although these are the first mRNA vaccines to be approved, the story of mRNA vaccines starts more than 30 years ago, with many bumps in the road along the way.

In 1990, the late physician-scientist Jon Wolff and his University of Wisconsin colleagues injected mRNA into mice,

which caused cells in the mice to produce the encoded **proteins**. In many ways, that work served as the first step toward making a vaccine from mRNA, but there was a long way to go—and there still is, for many applications.

Traditional vaccines use a weak or inactive form of a microorganism to turn the immune system against the disease. After a person is given injection of an mRNA vaccine, their cells make part or all of a protein that causes an immune response, including the production of antibodies. Although the most widely known examples

are the mRNA-based vaccines from BioNTech–Pfizer and Moderna directed against the SARS-CoV-2 coronavirus that causes COVID-19, that is just one small part of this field—and those vaccines were not the first efforts that used mRNA.

Despite the many benefits of using this molecule as the basis of a vaccine, it comes with fundamental challenges: it is not very stable inside cells, and mRNA is not efficiently translated into proteins when used as a gene-delivery tool. Today, mRNA can be engineered to battle many diseases, but it will not work with all of them.

## The upsides of mRNA

German biotechnology company BioNTech's chief medical officer Özlem Türeci—physician, immunologist and entrepreneur—says that “mRNA has a couple of interesting features that make it attractive for vaccines.” Adaptability serves as this molecule's key feature in this application and beyond. mRNA can be engineered not only to make antigens for vaccines but also to encode antibodies, cytokines and other proteins related to the immune system. “The versatility of mRNA creates a huge design space,” she explains.

The scientists at BioNTech spent years researching and developing techniques to get full command over mRNA, including optimizing its non-coding parts, designing specific sequences, developing manufacturing processes and more. Türeci describes the results of those efforts by saying, “We have a diversified toolbox and by mixing and matching the modules in this toolbox, we can design mRNA with the features that we need for a particular purpose.” She adds that “it is a bit like writing code—by mastering a programming language [that] is rich in terms, one can give any instruction one wants.” With the BioNTech toolbox, the scientists can control how much protein is produced and for how long, the route of administration of the mRNA, which cells express the protein and if the mRNA creates a precise activation or suppression of the immune system.

Once scientists know what mRNA they want to make, the process is relatively easy. For vaccines, using mRNA is much quicker than the traditional approach, in which the vaccine is grown in cells or in chicken eggs. To make mRNA, a scientist starts with a computer to lay out the desired sequence. Then, an *in vitro* transcription reaction is used to create a DNA template that can synthesize the desired mRNA. So, this process does not require cell culture or animal material, and the manufacturing process stays mostly the same regardless of the sequence of the mRNA.

## Enhancing the approach

Although the high efficacy of mRNA vaccines seems miraculous in the fight against COVID-19, that is far from the whole story. Wolff's work in the 1990s set off interest in using mRNA vaccines, but scientists ran into a fundamental problem: “RNA is highly inflammatory,” says physician-scientist Drew Weissman of the Perelman School of Medicine at the University of Pennsylvania.

In 2005, Weissman and his then-colleague Katalin Karikó—now at BioNTech—found a way to make RNA less inflammatory. They [showed](#) that the

inclusion of modified nucleosides, part of the basic structure of RNA, resulted in a dramatically lower inflammatory response. This work explored the use of nucleosides such as 5-methylcytidine, pseudouridine and other forms. With these modifications, Weissman says, “you could increase the amount of protein that mRNA could make by 10- to 1,000-fold and make a much better vaccine.” Plus, [chromatographic techniques](#) can remove contaminants, such as double-stranded mRNA, which results in an even lower inflammatory response.

A decade later, Niek Sanders—the principal investigator at Ghent University's laboratory of gene therapy and the scientific founder of Ziphys Vaccines—and his colleagues found a different modification for mRNA. mRNA that incorporated the N<sup>1</sup>-methylpseudouridine modification by itself or with 5-methylcytidine [produced](#) as much as 44-fold more of its intended product than mRNA with previous modifications produced, and it still resulted in a diminished immune attack on the molecules. “This is still the best modification, and it is also used in the COVID-19 mRNA vaccines of BioNTech–Pfizer and Moderna,” Sanders says.

## Constructing a carrier

Chemically modified or not, just injecting mRNA alone will not work. “Naked mRNA gets destroyed and [is] not taken up by cells,” says microbiologist Justin Richner of the University of Illinois College of Medicine at Chicago. Once the mRNA is injected, extracellular ribonucleases cut it up.

Various versions of lipids, such as [ionizable lipid nanoparticles](#), can be used to safely deliver the mRNA to target cells. Türeci and her colleagues optimized a therapy with what she describes as “different liposomal formulations to make RNA fit for the respective purposes like an intramuscular or intravenous injection and targeting specific cell types.” BioNTech found that for anti-cancer vaccines based on liposomally formulated mRNA, for instance, the antigen is expressed mainly in the dendritic cells in lymphatic compartments. These cells specialize in setting off antigen-specific immune responses.

In the future, scientists hope to have far more control over the resulting protein production. In a collaboration that included synthetic biologist Ron Weiss of the Massachusetts Institute of Technology and others, Sanders [described](#) switchable mRNA. “It's an on/off switch for mRNA,” Sander says, “and we proved that it works in mice.” With this form of mRNA, the therapy can be turned on when needed, and the level of protein production can be more precisely controlled.

Each of these improvements—less inflammation, increased expression, protected delivery and controlled protein production—allows researchers to build better vaccines based on mRNA.

## Improving vaccines against influenza

Among the most commonly used vaccines, the vaccine against influenza is perhaps in need of the most improvement. This vaccine is [estimated](#) to prevent tens of thousands of hospitalizations each year. However, [data](#) from the US Centers for Disease Control and Prevention on vaccines against seasonal influenza for 2009–2020 indicate an average effectiveness of about 43%. In this period, even the most effective vaccine, for 2010–2011, reached an efficacy of only 60%, and the worst case, in 2014–2015, it reached an effectiveness of only 19%, protecting about one in five people.

In defense of these vaccines, they must track a moving target. “Influenza vaccines are the only mass-distributed bioproduct that changes routinely,” says Philip Dormitzer, Vice President and Chief Scientific Officer: Viral Vaccines at Pfizer Vaccines Research and Development. “A big challenge with flu is keeping up with the changes.”

With traditional methods of making a vaccine against influenza, developers must modify the virus or protein being made. That modification can require changes in manufacturing. For example, the modified virus might grow a little differently than expected, which might require changes in a vaccine's formulation. Plus, vendors usually start making vaccines against influenza six months in advance of using them, so by the time people get the vaccines, they might not provide protection against the most prominent influenza strains of the season.

With an mRNA-based approach, Dormitzer says, “swapping one gene for another with mRNA changes its properties very little in manufacturing, which is much easier than changing a viral strain.” Speed also matters, and developers can quickly make mRNA vaccines. “The closer you can move the strain selection to flu season, the more accurate you will be,” Dormitzer says. By being able to make mRNA vaccines faster, manufacturers can select the influenza strains to target later than they are able to with traditional methods, which should increase the efficacy of the treatment.

The engineering behind mRNA vaccines also allows scientists to build multi-valent vaccines. “We can go up in the number of antigens being expressed,” Dormitzer explains, “which could increase the robustness of a flu vaccine.”

Seeking approval for a new vaccine against influenza, however, is different than

it has been for COVID-19, which had no treatment or vaccine. For influenza, there are a “number of vaccines out there, but their efficacy could be better,” Dormitzer says. “So, it’s very important that a flu vaccine check all of the boxes: efficacy, reliability, supply, tolerance and so on.”

Consequently, a pharmaceutical company is likely to market an mRNA-based vaccine against influenza only when it surpasses existing ones in several ways.

### Exploring other infections

COVID-19 and influenza are just two of many infectious diseases that might be treated with mRNA-based vaccines. For instance, Weissman says, “We are working on about 30 different mRNA vaccines, including ones for influenza, HIV, hepatitis C, malaria, tuberculosis and many others.” That alone shows how flexible mRNA can be for building vaccines.

One vaccine made from mRNA and lipid nanoparticles is very similar to another, Weissman notes. “The important thing is finding the right antigen,” he adds. “We spend a lot of time and work with lots of experiments to find the best antigen to make a vaccine work the best.”

Finding a good antigen to target is easier with some infections than with others. With HIV, Weissman says, “the envelope is the important antigen, but it mutates rapidly and it’s covered in sugar, and you need to address those issues to make an antigen that produces the right response.” Changes in the design of the mRNA might also be required.

Weissman and virus expert Harvey Friedman of the University of Pennsylvania found targetable antigens for genital herpes. Using these antigens, the scientists developed a vaccine from nucleoside-modified mRNA and lipid nanoparticles. Tests in mice and guinea pigs showed that this vaccine prevented infection with the virus that causes [genital herpes](#). “This vaccine is moving into clinical trials,” Weissman says.

The use of mRNA for vaccines also holds hope for previously intractable, but highly prevalent, infections with pathogens such as dengue virus. [Dengue virus](#), which is carried by mosquitoes, endangers nearly half of the world’s population and infects as many as 400 million people a year. Since there is no treatment for this infection, Richner is working on a vaccine.

“Dengue is somewhat complicated,” Richner says. It consists of four different viruses that cause a similar disease. “We want to target all four,” he notes. Targeting all four dengue viruses is necessary, as a subsequent infection with a different dengue virus tends to be more severe, due to antibody-mediated enhancement.

Richner and his colleagues started with dengue virus serotype 1. Like Weissman, Richner’s team used a nucleoside-modified mRNA in lipid nanoparticles. [Neutralizing antibodies](#) elicited by the vaccine were sufficient to protect mice against a lethal challenge. Now, Richner’s team is working on expanding this vaccine to serotypes 2, 3 and 4, and the differences in the dengue viruses require some adjustments in targeting each one. “We’ll need to optimize the vaccine for each virus,” he says. The goal is to provide protection against all four dengue viruses with one vaccine.

At CureVac, data from a [phase 1 clinical trial](#) of the company’s mRNA-based vaccine against rabies looks promising. “A very low dose vaccination generated an immune response in all subjects,” says Thorsten Schüller, CureVac’s vice president of communications. “This demonstrated the potential of our mRNA technology for the first time.”

### Creating vaccines against cancer

Before COVID-19 hit, Türeci and her colleagues at BioNTech were working on mRNA-based vaccines against cancer. “You want to confront a patient’s immune system with a wanted poster of the enemy and train the immune system’s effectors to recognize the enemy and teach the immune system that this is dangerous.”

Türeci says that mRNA can be used to deliver two types of cancer antigens. The first approach is to present to the immune system a person’s own antigens that are usually shut down in healthy cells—antigens encoded by embryonic genes would be an example of this—but are expressed by the cancer. Here, an anti-cancer vaccine would trigger an attack on cells carrying those antigens. “For each cancer indication, we use computer algorithms and machine learning to identify the antigens that cover as many patients as possible.” For melanoma, as an example, four antigens cover more than 90% of the patients. [BioNTech](#) made a multi-valent RNA-based vaccine that targets all four antigens and is in clinical trials.

Alternatively, an mRNA-based vaccine can target a cancer’s mutations. The profile of mutations, however, is unique to each patient, and that requires a personalized approach. “This is the perfect playground for mRNA,” Türeci says. “We start from a patient profile, generate a multi-valent, multi-mutation vaccine in four weeks for this patient and treat them with it.” This method, which is in several clinical trials run by BioNTech and Genentech/Roche, uses a approach similar to that used for making the BioNTech–Pfizer vaccine against COVID-19. Türeci describes the strategy as analyzing

“genetic information to tailor a vaccine and manufacture it fast.” She adds, “We had already done that hundreds of times for our cancer patients,” and that explains some of the speed behind the development of their vaccine against COVID-19 and why she and her colleagues feel prepared to adapt to viral variants, if necessary.

For solid tumors, an attack by the immune system is not enough. The tumor’s microenvironment fights off the immune response in various ways, including suppressing the actions of T cells. For melanoma, says biophysicist Leaf Huang of the University of North Carolina at Chapel Hill, “the tumor microenvironment is the real barrier for these vaccine treatments.” A vaccine must be combined with another treatment that modifies that microenvironment, allowing the vaccine-triggered T cells to enter the tumor tissue. [Huang and his colleagues](#) combined a vaccine with the chemotherapy sunitinib and found that this combination helped immune cells reach the tumor and thereby increased the efficacy of the vaccine. Cytokines such as IL-12 are also good candidates for breaking the immunosuppressive tumor microenvironment, according to Sanders, whose team [successfully combined](#) IL-12 gene therapy with a gene-based anti-cancer vaccine.

Nonetheless, Huang says, “The development of agents that can be used safely and effectively to modify the tumor microenvironment still has a long way to go.”

### Expanding innovation

In many ways, mRNA vaccines are just getting started. “We do not have a platform for every disease, but the great advantage of mRNA vaccines is that we can test novel hypotheses in rapid succession,” Richner says. “For new vaccines, we need to find what makes a good immune response, and that requires basic science.”

This field will drive more basic science for years. Plenty of engineering will be involved, as well. At BioNTech, Türeci calls the company’s vaccine scientists “immune engineers,” and she envisions many advances ahead. As she thinks of the future possibilities for mRNA vaccines, she says, “It’s about the nature of innovation—not one invention, but finding out what is possible in many things and bringing them together.” □

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