The Vaccine Trenches

Key breakthroughs leading to the powerful mRNA vaccines against COVID-19 were forged at Penn. That triumph was almost 50 years in the making, longer on obstacles than celebration, and the COVID-19 vaccines may only be the beginning of its impact on 21st-century medicine.

By Matthew De George

Katalin Kariko is most comfortable at a lab bench. That's where she's spent most of a career that dates to the late 1970s. Even as a lead researcher on papers that broke new ground in gene therapy, even as a vice president of an emerging biopharmaceutical power, she was never above doing the unglamorous work in the lab: the repetitive cell culturing and data collection that she easily could have delegated.

Lately her time in the lab has been reduced by the COVID-19 pandemic, along with a shift to a more advisory role at BioNTech, the German pharmaceutical company she joined in 2013. (She remains an adjunct professor at the Perelman School of Medicine.) But the diligence that served her so well in the lab still shines through as she reviews the path she's taken through five decades of science research.

Speaking from her home office in the Philadelphia suburbs in February, Kariko—Kate or Katy to her colleagues—strikes a tone most remarkable for its magnanimity. When you ask about the messenger RNA (mRNA) technology behind the first two commercially available COVID-19 vaccines to hit the American market—technology revolutionized and refined at the Perelman lab in which Kariko worked for decades—you don't get any self-adulation. Nor do you get any resentment at the many entities that turned down her research funding requests—no matter how justifiable a bit of bitterness might be.

Instead, you get a tour through the history of all the stepwise improvements, from Kariko's lab and collaborators across the world, that have led us to the current moment, in which mRNA offers

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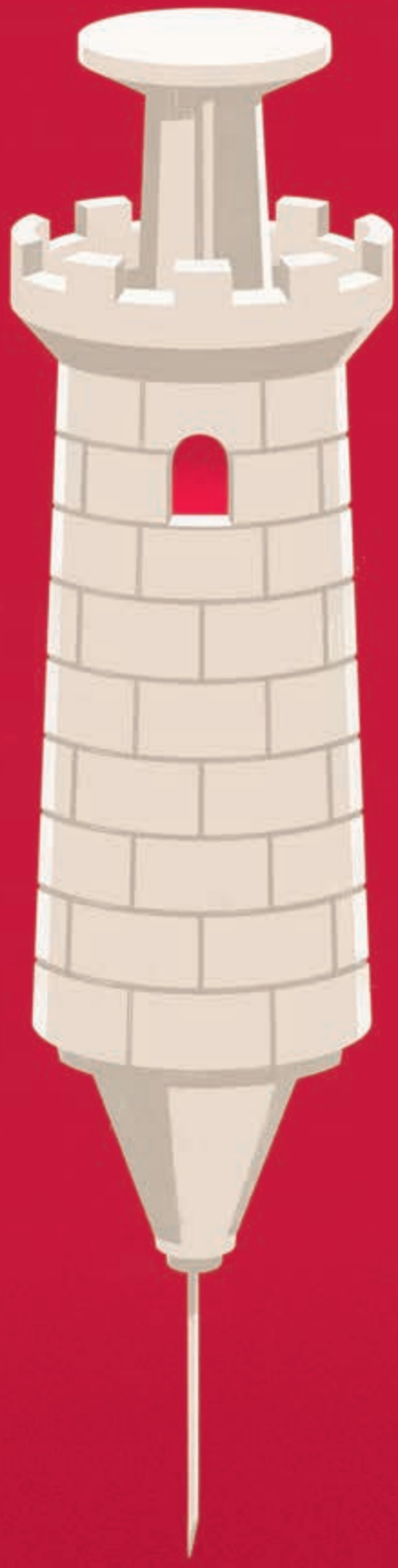

not just a chance to stanch a global pandemic but also a promising new platform to tackle all manner of diseases.

Though she's not above enjoying the acclaim she has lately received for the technology she's long championed, Kariko quickly puts her contributions to it in perspective.

"Science builds on science," she says. "We always built on the people who came before us, and people will use our data. Of course, everything was important that those people did. I would hug them if I could."

Kariko's attitude is a key part of how she and others helped unlock the promise of a method that many dismissed as a clinical dead end in the 1990s. When the sector struggled to attract funding—when Kariko's attachment to it imperiled her job prospects at Penn—she and her Perelman colleague Drew Weissman, a professor of infectious diseases, battled to defend its merits. They persevered in part because of their openness to trying new things, to share their findings and entertain new possibilities even when their ideas were met with skepticism.

The end product is a pair of safe and effective vaccines, produced by Moderna and a partnership between BioNTech and Pfizer, that have sped to market in record time. They are based on mRNA, which codes for a molecular analogue of the spike protein that lines the SARS-CoV-2 virus, and uses that analogue to teach the immune system to develop antibodies against the virus. Yet this technology isn't just a powerful weapon against the pandemic. The underlying method represents a new frontier in biologic medicine whose vast possibilities encompass infectious diseases, cancer treatments, and even repairing autoimmune and genetic conditions.

"We knew when we started with this technology that it would be very useful if a pandemic hit, because it's so fast and so easy to make a vaccine with it," Weissman says. "But we weren't hoping for a pandemic to prove that."

Weissman's journey to mRNA started with a photocopy machine, where he used to jockey for pole position with Kariko. At least that's what he likes to joke. Even if they hadn't spent so much time in line to run off pages from academic journals, Kariko is certain she would've found Weissman sooner or later.

The basic science of ribonucleic acid (RNA) was well established by the time Kariko arrived at Penn in 1989. Discovered in 1961, it plays a foundational role in just about all forms of life, enabling cells to synthesize proteins encoded in their genome. In human cells, the genetic code rests in double-stranded DNA (deoxyribonucleic acid). Genes are transcribed in the nucleus to messenger RNA (mRNA), which

Photograph by Candace diCarlo

travels into the cell's cytoplasm and is translated into proteins in the ribosomes. Some viruses, including SARS-CoV-2, use single-strand RNA instead of DNA as their only genetic information.

Kariko arrived at Penn during what she calls "a revolution" for mRNA science. She was hired by Elliot Barnathan C'77 M'81 GM'86 GM'87, then an associate professor of medicine, to conduct basic research in his lab, which focused on blood vessels. Even in that position, Kariko was always looking for ways to incorporate mRNA, either as a remedy or part of the investigation process. Excitement in the field was growing. In 1985, the first polymerase chain reaction (PCR) machines, which allowed scientists to customize strains of RNA, were patented. (Even three decades later, Kariko describes that new technology as "so empowering.") Another key advance was the development of positively charged lipids that could encase and deliver negatively charged RNA to cells, a technology made commercially available in the late 1980s. By 1990, the Human Genome Project added attention to the field of genetics, though it shifted funds toward DNA research. Despite that, a proof of concept study of synthetic mRNA translation in living mouse models was published in 1990, and the first therapeutic use of RNA in rats was reported two years later.

Kariko and Weissman helped unlock the promise of a method that many dismissed as a clinical dead end in the 1990s.

Photograph by Candace diCarlo

Those findings ran against the prevailing wisdom that because mRNA too easily degraded in the body, it wouldn't have therapeutic value.

Kariko traces her interest in mRNA back to her first day in the lab in her native Hungary in 1978, when her graduate supervisor tasked her with collecting a sample of RNA that could be shipped to a lab in New Jersey for sequencing. She was drawn to the potential power this class of molecules might have in medicine. If you could tailor mRNA to inject into cells, you could control which proteins they produce, what genes they express, what metabolic pathways they follow. Her zeal earned her a reputation as "the RNA hassler," at Penn and beyond. "I went to meetings and if someone was sitting next to me, I'd ask what they were

doing and I always offered, 'Oh I can make an RNA for you,'" Kariko recalls.

"Kate was really just unbelievable," says Barnathan, who is now the executive director for research and development at Janssen, a pharmaceutical company under the Johnson & Johnson umbrella. "She was always incredibly inquisitive. She read voraciously. She would always know the latest technology or the latest paper, even if it was in a totally different area, and she'd put two and two together and say, 'Well why don't we do this?' Or, 'Why don't we try this formulation?'"

That enthusiasm paid off as funding dried up in the 1990s. At that time, mRNA began to fall out of favor. Since mRNA interventions couldn't modify the genome the way DNA therapy theoretically could, they were seen as short-term aids, not the moonshot solution to thorny problems like hereditary diseases that attracted much of the funding.

Kariko was demoted from the tenure track in the mid-1990s, from a research assistant professor position to a newly created role as senior research investigator. When Barnathan left for the private sector in 1997, she joined the neurosurgery lab of David Langer C'85 M'90 GM'98 for two years ["Alumni Profiles," Jan|Feb 2020]. But even as she bounced around between increasingly tenuous perches, she continued publishing, including a 1999 paper (based on research done in 1996 with Barnathan) in which she used urokinase receptor proteins to demonstrate effective overexpression of in vitro transcribed mRNA in living cells.

Weissman arrived at Penn in 1997, having spent seven post-doctoral years at the National Institutes of Health. In a lab run by Anthony Fauci, Weissman explored the role of dendritic cells, one of the "sentinel cells" that detect and help defuse threats to the immune system, in HIV infection. At Penn, his research focused on dendritic cells' broader response to pathogens.

As he dug into the literature, Weissman spent countless hours xeroxing pages from academic journals … which meant a lot of time waiting in line with Kariko. The more they chatted, sharing tidbits of their research, the more "the RNA hassler" made Weissman wonder if mRNA could be useful in his lab. Soon they were collaborating.

The pairing proved to be kismet, and while the infusion of funds from Weissman's new lab didn't solve all the financial quandaries, it opened up new avenues for Kariko's explorations. They endured frustrations along the way. Kariko had little success in getting grants or interest from venture capitalists. At times it seemed like the powers-that-be were rubbing her nose in it. In the early 2000s Kariko and Weissman were approached by a pair of MBA students competing in a Wharton School entrepreneurship competition, but the idea of a company built around mRNA technology was, according to Kariko, deemed too implausible by the contest's board.

"Katy and I worked on this from the beginning," Weissman says. "We never gave up. We never felt that it was bad technology and we had to stop. But we

Bringing the Vaccine to the Masses

Mace Rothenberg C'78 offers a rueful laugh when recalling his 2020 calendar. As Pfizer's chief medical officer, his duties included traveling to the company's many labs and research stations around the world—a task once done in person, no Zoom required. On his itinerary for the first week of March: a trip to meet teams at Pfizer's research and development center in … Wuhan, China, where COVID-19 was first identified.

That trip never happened. By March, Wuhan was starting to see the light at the end of a months-long tunnel of lockdowns. The United States was staring into the abyss, with schools closed and businesses shuttered. Rothenberg's check-ins with employees, in Wuhan and elsewhere, shifted from the usual to the extraordinary, emphasizing measures to keep employees safe without disrupting a global supply chain of medications, COVID-19-related and otherwise.

By January, Pfizer CEO Albert Bourla decided to put Pfizer out front in the vaccine quest, marshalling resources throughout the pharmaceutical giant.

"It was really a test of the organization's strength and culture," Rothenberg says. "We talk about culture a lot, but it's not until you're really challenged by something unexpected that you see what the characteristics and the culture of an organization are. Looking back at the last year, it's really extraordinary. I feel really privileged to be part of an organization that responded so well to this challenge."

Rothenberg joined Pfizer in 2008 and spent 10 years in its oncology department, rising to chief development officer of oncology in 2016. He became CMO on January 1, 2019, a position he held until his retirement in January 2021. (He'll remain with the company until the spring and will then shift to an advisory role.) Having done most of his clinical research in cancer, he wasn't well acquainted with mRNA vaccinology before the pandemic. Like everyone at the company, he got a crash course as Pfizer partnered with BioNTech, the Germany-based firm founded by the husband-and-wife team of Ugur Sahin and Ozlem Tureci.

One thing Rothenberg was familiar with was Pfizer's potential to play a key role in coordinating a complex endeavor. In pulling together a company-wide effort, Rothenberg relied on a key standby of his history: collaboration. One of his most significant projects in oncology research was helping to create Project Data Sphere, a digital "library-laboratory" that allowed scientists to share in-process and pre-published cancer research. Launched in 2014, it has allowed researchers to share ideas, leading to more than 100 novel datasets and more than 20 peer-reviewed papers.

had to fight the entire way. We spent time trying to get people, biotechs, and pharmaceuticals interested in this. It took a long time, but now we've achieved it."

Despite those setbacks, the work continued. Kariko and Weissman improved the technology by conquering one obstacle at a time.

One major issue was RNA's inherent toxicity. Free-floating RNA outside of cells is usually a sign that something is wrong, like rupturing cells or viral intrusion. Its presence triggers an inflammatory response, and the space between cells is full of enzymes that rapidly degrade RNA. Its short half-life outside of cells was a major impediment to overcome.

By 2004, the pair had worked out which receptors their therapeutic mRNA tripped. Kariko noticed that a different type of RNA, transfer RNA, rarely triggers the immune system. She theorized that it slipped under the radar due to modifications in the nucleosides that comprise it. By tinkering with the nucleosides in her synthetic mRNA, Weissman and Kariko dampened the unwanted immune response, a process they patented in 2005 and have licensed to companies including Moderna. (BioNTech, which Kariko joined in 2013 as a senior vice president, funds research at Weissman's lab.)

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From afar, Barnathan sees the totality of Kariko's work in this field as a "patchwork" of little solutions, that when stitched together produced an innovative whole.

"She would take those pieces of information, put it all together, and she wanted a pretty thing that would keep you warm and was beautiful, and that's what her mRNA was," Barnathan says. "It was a patchwork quilt of putting together little scientific discoveries here and there—some which had been discovered a long time ago, some that were just published in *Science* the day before. But she would take that information, put that together, and keep iterating on how to do it."

The nucleoside breakthrough thrust the technology into the fast lane. In recent years it has been deployed widely, though most treatments remain in clinical trials, which can take up to a decade or more to complete. Moderna in 2010 used it to regenerate pluripotent stem cells, and there is growing recognition that mRNA-based techniques could open a new frontier in gene editing that could complement the power of CRISPR-Cas9, the DNA-editing method that was honored with the 2020 Nobel Prize in Chemistry. In 2014, Kariko joined BioNTech cofounders Ugur Sahin and Ozlem Tureci to author a paper hailing mRNA as "a new class of drugs." An article by Weissman and Kariko the next year

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The same tack proved useful for COVID-19. Pfizer was transparent with its protocols for clinical trials, releasing them to the public in September. The company shared preliminary results, providing a window into the process and inviting feedback from colleagues. Like other institutions, it used pre-print servers, which publish scientific papers before peer review, to permit promising information (and, Rothenberg concedes, some bad data) to reach scientists faster than the usual publication process.

Collaboration has been vital on the distribution side, too. Pfizer's global president of vaccines is Nanette Cocero Gr'93 WG'94. In her 15 years at Pfizer, she's worked all over the world, formerly leading the emerging markets division of Pfizer's Innovative Health business. She's also the chair of the Vaccine CEO Steering Committee of the International Federation of Pharmaceuticals and Manufacturers Association (IFPMA), which sets industry-wide best practices on access and affordability. IFPMA was an early participant in COVAX, a joint effort by the World Health Organization; the Coalition for Epidemic Preparedness Innovations; and Gavi, the Vaccine Alliance. Against a virus that knows no borders, global cooperation and coordination are vital.

"The spirit of collaboration that we have seen in this pandemic is something I hadn't witnessed before but that I hope will continue long into the future," Cocero said in an interview with *Wharton Magazine*. "As an industry, we committed to work as one team to harness our scientific expertise, our technical skills, and our manufacturing capabilities to do everything in our power to get life-saving breakthroughs into people's hands as quickly as possible."

Like Penn mRNA researcher Drew Weissman [see main story], Rothenberg has taken a public advocacy role. Pfizer launched a series of public-service announcements through the "Science Will Win" campaign, including videos of Rothenberg laying out the vaccine's benefits in layman's terms. His physician's bedside manner evident, Rothenberg says he approached it as though talking to his mother. It's hardly conventional marketing—vaccine contracts come through governments, so Pfizer isn't competing for consumer market share, per se, but rather competing against misinformation—but then this isn't a conventional challenge.

"I think more than for most other products, there really was a need to educate people because this was a new platform, this was a disease that people didn't know about, and we didn't have long-term follow-up on the vaccine or even the disease," Rothenberg says. "We're now learning more about that as we go along," and at each step "educating the public about the disease, about protective measures you can take, and about how the vaccine was developed—and addressing some concerns and misinformation that's out there regarding an mRNA platform."

billed mRNA as "fulfilling the promise of gene therapy," a technology coming "out of the shadows and into the spotlight," as Kariko wrote. In 2017, Weissman and his colleague Norbert Pardi, a research assistant professor of medicine, hit a significant milestone in the production of a single-dose mRNA vaccine for Zika virus that was effective in mice and rhesus macaques. Pardi has since turned his attention to other pathogens, including mRNA-based influenza vaccines.

"We had the platform, we just needed to figure out what we wanted to do with it," Pardi says. "It's extremely versatile. We can use it for many, many purposes."

Weissman says that when he first heard of the novel coronavirus causing severe respiratory disease in Wuhan, China, his mind didn't automatically jump to mRNA. And for all her decades of promoting the therapeutic possibilities of mRNA, Kariko didn't either. "When I heard about it in February, I thought, 'Oh, it is in China; it won't get here,'" she recalls. "But the CEO of BioNTech, Ugur Sahin, he's a visionary, and he immediately thought, 'Oh, we need to do something with that.'"

These days Weissman spends much of his time outside the lab, talking to non-scientists about COVID-19 vaccines that use mRNA. In those conversations, he walks a fine line. Yes, the vaccine came together with unprecedented speed—less than a year compared to the more typical timeline of up to a decade. But no, the technology isn't new. Only the application is.

"People say, 'I'm afraid of this vaccine; it was developed 10 months ago,'" Weissman observes. But he is quick to answer: "It wasn't. We developed modified RNA 15 years ago."

The mRNA technology is ideal for the challenge: quick, safe, and effective. It is produced in a cell-free system, transcribed in vitro from enzymes and a genetic template in a few hours. There was nothing that physically needed to be transported (i.e., cell cultures or samples) from scientists at the beginning of the outbreak. "A year ago, a message came over the internet and we could learn what is the sequence of the virus," Kariko said during a Perry World House panel in January. "If it had happened 20 years ago, you had to have physically the sequence in your hands, the viral construct in your hands. But here, the information was sufficient."

The vaccines produced by Moderna and BioNTech/Pfizer have two components: mRNA, cased in lipid nanoparticles (LNPs). The latter is a delivery device, an extra layer of cloaking that helps localize delivery without sparking an unwanted immune response. The mRNA encodes a protein analogous to the spike proteins that protrude from the SARS-CoV-2 virus and allow it to bind to and infect cells. Dendritic cells take up the mRNA, translate it to proteins that are incorporated into the cell membrane, and present these engineered products to the body to induce an adaptive immune response. The process produces antibodies that remain in the bloodstream so that if a vaccinated individual encounters SARS-CoV-2, a neutralizing response can quickly be mounted. (Both vaccines encode identical spike proteins; their proprietary mRNAs differ slightly in the non-coding regions and utilize different lipids.)

mRNA vaccines have a lot going for them from a safety perspective. Since they don't contain any of the pathogenic substance—seasonal flu vaccines, for instance, use heat-killed or weakened virus, which is why a small percentage of people can get sick from them—there's no chance of contracting COVID-19 from the vaccine. The therapeutic mRNA in the vaccine cannot penetrate the nucleus, so it can't alter the genome. The vaccines produce a relatively robust and durable immune response. Studies as of early 2021 indicate that the vaccine still protects against some of the newly emerged variants of SARS-CoV-2, though they show a "small but significant" reduction in efficacy.

While many companies are pursuing COVID-19 vaccines by different methods, the first two to hit the market utilize mRNA. The Pfizer/BioNTech vaccine began its Phase 3 clinical trial last July and received emergency use authorization from the US Food and Drug Administration on December 11. A week later, Moderna got the green light. (Three other vaccines in various states of development and approval, from AstraZeneca/Oxford, Janssen/Johnson & Johnson, and Novavax, utilize other technologies, some of which offer advantages including longer shelf life and less stringent storage temperature requirements.)

"It was with bated breath that we watched the development of this vaccine," said Paul Offit, the Maurice R. Hilleman Chair of Vaccinology at the Perelman School of Medicine, at the same Perry World House panel. "And both the Pfizer and Moderna products have been remarkably successful, at a level I think no scientist would have predicted a year ago."

There are some limits to what we know about these COVID-19 vaccines, mostly because we've had a limited time to observe their effects. The trials for both versions passed the required safety hurdles, but questions remain about long-term effects for certain population subgroups, like pregnant women. Clinical trials on children are also just getting off the ground. These questions can't be answered until there's been more time to study them. The same can be said of the virus itself, which humans have only known for a little more than a year.

Yet the mRNA vaccines have already achieved an impressive track record, with more than 60 million doses dispensed by mid-February. They are 95 percent effective at blocking infection and 100 percent effective in blocking severe infection.

Vaccines, Offit emphasizes, involve a risk/reward calculus. The risks of a virus that has killed millions are known and dire. The risks of the vaccine remain largely theoretical, as a full understanding of rare side-effects in certain populations will simply

take more time to develop. "When you look through all the data, you can't help but be compelled by the safety and efficacy of this vaccine," Offit said. "The choice to get a vaccine is an easy one right now."

When Weissman and Pardi presented a paper in 2017 on an LNP-encapsulated mRNA vaccine method, recent human history was on their mind. Twice in the previous two decades, coronaviruses had caused respiratory viruses that reached epidemic status: SARS in 2002, MERS in 2012. Taking the long view, it was not a matter of *if* another pandemic would happen, but *when*. While they were focused to some degree on the "renaissance in the field of therapeutic protein delivery" that mRNA offered for conditions ranging from cancer to hereditary genetic diseases, Pardi in particular had a growing understanding of how the mRNA technology could be mobilized in the face of an infectious disease.

Even in the midst of the COVID-19 pandemic, that perspective still applies. If the past is any guide, there will be another pandemic sometime in the future caused by a virus like SARS-CoV-2, maybe one deadlier than the current crisis. The technology that informs the COVID-19 vaccines presents a powerful weapon in the arsenal for when that day comes.

"The technology really is limitless," Weissman says. "There are thousands of diseases, there are hundreds of vaccines that we could make, many of which we're working on. A lot of it is how to pick, and we pick based on how important the disease is and how doable it is."

Economic incentives play a role—and that dynamic has now flipped. From a technology for which Kariko once struggled to obtain thousands of dollars in funding, mRNA therapy is now a multi-billion-dollar industry. AstraZeneca, for instance, paid Moderna $240 million in 2012 for drugs that hadn't yet been developed. Moderna was valued at $7 billion when it went public in 2018— with the NASDAQ stock symbol MRNA—before its first treatment had been approved. Its market capitalization is now north of $50 billion. BioNTech's market value has grown from $3.39 billion at its initial public offering in October 2019 to more than $26 billion in January 2021.

Kariko's current research deals primarily with cancer treatments, including a partnership with the pharmaceutical company Sanofi. BioNTech is also exploring treatments for the autoimmune disease multiple sclerosis, which has showed promising early results. Weissman's lab at Penn has five mRNA vaccines in trials, covering seasonal flu, HIV, and herpes. Weissman has funding from the Bill and Melinda Gates Foundation to pursue a possible single-injection remedy for sickle cell anemia, an mRNA therapy that would target bone marrow stem cells to rewrite the area of the genome encoding aberrant proteins.

Even within the narrow lane of infectious disease, the scale is mind-boggling. The World Health Organization has identified around 150 infectious zoonotic viruses, those that have jumped from animals to humans. Estimates of the reservoir of potential zoonotic viruses in mammals vary widely, from around 10,000 to half a million. Any one of them may have the potential to mutate and jump from animals to humans like SARS-CoV-2 did.

It's possible that the next blockbuster use for mRNA is a disease unknown to science. But the technology's simplicity and scalability offer a plug-and-play platform promising drug development in a fraction of the time required by traditional methods.

"I think governments and policymakers also need to learn the lesson that they should support basic science, and they should support vaccine development prior to a pandemic because it's too late to develop vaccines in the middle of a pandemic," Pardi says. "You need to develop these vaccines before a pandemic. We know many of the viruses and other pathogens that can potentially cause an outbreak and can potentially cause the next pandemic, and we have to be ready for those events. And the way to be ready is to develop vaccines prior to those outbreaks. And the messenger RNA technology is really fantastic because if you develop these vaccines, these prototype vaccines, and if you have an outbreak and maybe the virus that causes the outbreak is slightly different from the vaccine strain, you can use the technology to very quickly adjust the vaccines."

The technology offers a plug-and-play platform promising drug development in a fraction of the time required by traditional methods.

Those challenges lie in the hypothetical future. The pathway to get there with the technology, if history is any guide, is to chip away at problems sequentially. For now, mRNA's potential is being showcased globally at a critical moment, even as many additional innovations are being tested. The arduous journey from theory to application is a rewarding one for those who have shepherded the technology.

"It's very important to know that what I'm working on is something useful," Pardi says. "And now, we see that the work is very useful … and we hope that we can come up with more RNA therapies in the future and help even more people—not just vaccines but also other kinds of medicines. It's a really fantastic feeling, and this is what keeps us moving forward."

"It's a fantastic feeling," Weissman adds. "I'm a clinician, so my dream was always to develop something that would make people better. And I think we've done that."

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