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Delivering the message: How a novel technology enabled the rapid development of effective vaccines

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This year's Lasker∼Debakey Clinical Research Award honors Katalin Karikó and Drew Weissman for the development of a therapeutic technology based on nucleoside-modification of messenger RNA, enabling the rapid development of the highly effective COVID-19 vaccines.

By March of 2020, people across the world were slowly coming to the realization that their daily lives were about to change. A deadly pandemic was circling the globe. Other than the identification of a coronavirus as the likely culprit, we lacked knowledge of and immunity to the virus and had neither therapeutics nor vaccines to combat its spread and save lives. As we write this article, the coronavirus disease 2019 (COVID-19) pandemic is thought to have caused the death of 4.3 million people and sickened 208 million, and this is surely an underestimate. Due to an extraordinary feat of rapid vaccine design, development, manufacture, and distribution, just 15 months after the onset of the pandemic, the global community is gaining some semblance of traction, albeit progress is geographically uneven. Novel and highly effective vaccines based on nucleoside-modified messenger RNA (mRNA) encapsulated in lipid nanoparticles, designed and developed at breakneck speed, have become the major factor in controlling the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). How was it possible to create these novel vaccines in less time than any other vaccine in history? Where did these vaccines come from and what was the foundational science on which they are based?

One of the challenges that we in the scientific community face is communicating to the public how basic research benefits society at large. How do years of inquiry devoted to seemingly obscure problems contribute to the public good? Every once in a while a discovery is made that in the fullness of time has a truly obvious and dramatic impact on humankind. Such is the case for the groundbreaking contributions of Katalin Karikó and Drew Weissman who have been honored by the 2021 Lasker∼DeBakey Clinical Research Award. Their sustained efforts in adapting mRNA as a platform for producing therapeutic proteins in host cells are what enabled fast development of highly effective vaccines against the pandemic-causing SARS-CoV-2. These vaccines are having, and already have had, a major impact on the health of many millions of people around the world.

Karikó was born in Hungary where she obtained her PhD in biochemistry. In 1985, she moved to the United States as a postdoctoral fellow at Temple University in Philadelphia, where she began her long journey in adapting mRNA to therapeutic use, carrying out clinical research based on triggering interferon production with double-stranded RNA. As a research assistant professor at the University of Pennsylvania School of Medicine (now called the Perelman School of Medicine), she focused her efforts on using *in vitro*-synthesized mRNAs encoding therapeutic proteins for treating acute diseases. Even as Karikó faced many obstacles in her initial efforts at adapting synthetic mRNAs for use in cell cultures, she was unable to gain funding for the work and was demoted from her faculty position to senior research investigator. She exhibited remarkable resilience and persevered in her pursuit of an effective mRNA therapeutic. The game-changer was a fortuitous encounter with a newly arrived immunologist, Drew Weissman, who had received his MD and PhD from Boston University in 1987. Weissman came to the University of Pennsylvania in 1997 from the National Institute of Allergy and Infectious Diseases, where he had trained as a fellow (later a senior staff fellow) under the supervision of Anthony Fauci who, in an unexpected coincidence, would become a principal spokesman on the control of the COVID-19 pandemic in the United States. As colleagues at the University of Pennsylvania School of Medicine, Karikó and Weissman discovered they had a common interest in the delivery of synthetic mRNA to generate highly targeted therapeutic proteins. Although work on delivering therapeutic proteins using mRNA had been going on for decades in multiple labs, Karikó's and Weissman's complementary skills and expertise led to the breakthrough that enabled the rapid development of a vaccine for SARS-CoV-2.

Early on, Karikó and Weissman focused on using *in vitro*-synthesized mRNAs to direct the production of therapeutic proteins by delivering the mRNAs exogenously into cells in culture with cationic lipids. A critical obstacle they encountered was that synthetic mRNA was highly immunogenic in human dendritic cells of the innate immune system, triggering the synthesis of proinflammatory cytokines (Ni et al., 2002). As early as 2000, Karikó and Weissman published that transfection of dendritic cells with mRNA for the HIV polyprotein Gag induced a potent immune response, primarily through recognition of double-stranded regions of the RNA. In 2004 they reported that small interfering RNAs (siRNAs)

targeting viral genes during herpes simplex virus infection compromised therapeutic effects by activating Toll-like receptor TLR3 (one of the basic signaling receptors of the innate immune system) to induce type 1 interferon and enhance RNA degradation (Karikó et al., 2004). Several other research groups that had tried to use siRNAs as therapeutics also encountered the problem of RNA instability, and two groups (Diebold et al., 2004; Heil et al., 2004) reported that single-stranded RNA was recognized by Toll-like receptors.

A key early observation was that whereas synthetic RNAs triggered proinflammatory cytokine production, some naturally occurring RNAs, such as transfer RNAs (tRNAs), which were used as a control, did not (Koski et al., 2004). tRNAs are known to be rich in modified nucleosides (up to 25% of the nucleosides in tRNA are modified). The penny dropped when Karikó and Weissman realized that modified nucleosides might camouflage RNA from the innate immune system. In a seminal 2005 paper in *Immunity*, Karikó and Weissman reported that mRNAs synthesized *in vitro* using T7 RNA polymerase and a wide variety of modified nucleotide substrates reduced production of proinflammatory cytokines from monocyte-derived dendritic cells. Notably, uridine derivatives (such as 5-methyluridine, 2-thiouridine, and pseudouridine) abolished the capacity of mRNA to activate primary, blood-derived dendritic cells (Karikó et al., 2005). mRNA-mediated immune stimulation was suppressed proportionally to the number of modified nucleosides present in mRNA but even the presence of a few modified nucleosides exerted a suppressive effect. In what proved to be prescient, the authors noted at the end of their 2005 paper that the results of their study would be essential to the "design of therapeutic RNAs." Importantly, in later work, Karikó and Weissman went on to show that incorporation of pseudouridine (henceforth Ψ) not only reduced immunogenicity but also significantly increased translation of synthetic mRNAs both *in vitro* and in mice (Karikó et al., 2008). *In toto*, these results showed that modified mRNA could be used to produce high levels of proteins of interest without triggering strong inflammatory responses. Also, and at about the same time, Barry Polisky, founder of Sirna Therapeutics, made siRNA that was chemically modified (in which the 2′-hydroxyl groups were substituted with methoxy or fluoro residues) to increase the stability of the RNA. These investigators showed that when the modified siRNA was encapsulated in lipid and then introduced into a mouse model for hepatitis B virus infection, the alterations to the 2′-hydroxyl groups lowered cytotoxicity and immunostimulatory side effects (Morrissey et al., 2005).

The early publications from Karikó and Weissman garnered limited attention, but were noticed by a former young faculty member at the Harvard Medical School, Derrick Rossi. Rossi, who was studying stem cells, came across the Karikó and Weissman 2005 paper and used its findings to great success in his own research, delivering the four Yamanaka factors into adult human cells to achieve efficient reprogramming into induced pluripotent stem cells (Warren et al., 2010). Rossi's accomplishments based on using synthetic mRNAs with modified nucleosides did attract a lot of attention. He sought and received venture funding for a startup company named Moderna (for "modified RNA"), which was founded in the United States in 2010, with the goal of developing mRNA therapeutics. Moderna licensed patents that had been filed by the University of Pennsylvania based on the discoveries of Karikó and Weissman. (Rossi left Moderna in 2014 to focus on other entrepreneurial activities.) Meanwhile, another start-up company, BioNTech, which was founded in Germany in 2008 by Uğur Şahin and Özlem Türeci, also embraced nucleoside-modified mRNA, licensing the patents to the Karikó and Weissman technology. BioNTech's goal was to create a vehicle for a personalized cancer vaccine using specific tumor antigens. By 2015, BioNTech, where, at this point, Karikó had been appointed as a senior vice president, had expanded their original focus from developing vaccines for cancer immunotherapy to viral vaccine development. Meanwhile, Moderna, in 2013, had begun using Karikó's and Weissman's modified mRNA technology to create vaccines for Zika, chikungunya, and cytomegalovirus, as well a passive immunization using monoclonal antibodies.

The ability of BioNTech and Moderna to adapt mRNA as a vaccine against viral infections came only after years of work demonstrating that non-inflammatory, nucleoside-modified mRNAs could be translated at high levels in cells in culture and in mice, and, most importantly, the demonstration that the newly developed platform could be used therapeutically. By 2012, Karikó and Weissman reported that mice injected with lipid-complexed with *in vitro*-synthesized, nucleoside-modified mRNAs (in which uridine was replaced with Ψ) encoding erythropoietin elicited the production of elevated serum levels of the hormone as early as 6 h after injection (Karikó et al., 2012). This increase in erythropoietin levels was accompanied by a significant increase in reticulocytes and hematocrits. Similar results were obtained with non-human primates. The same coding sequence with unmodified uridine produced 10- to 100-fold lower levels of erythropoietin. During 2017 and 2018, Karikó and Weissman, working with postdoctoral fellow Norbert Pardi (who was later to join the faculty of the University of Pennsylvania School of Medicine), published a series of seminal papers demonstrating the use of modified mRNA as an anti-viral vaccine in mice and non-human primates. Protection against Zika virus infection was obtained by a single injection of lipid nanoparticles with 1-methylpseudouridine (m1Ψ)-containing mRNA encoding a virus antigen (Pardi et al., 2017). The lipid nano particles enabled fusion with the host cells and also proved to be an effective adjuvant. The vaccine elicited a potent and durable neutralizing antibody response to the Zika envelope glycoproteins. Because the Zika virus epidemic occurred in 2013, and the success of this vaccine candidate was not reported until 2017, human trials were not possible as by then the epidemic had abated and there were too few susceptible individuals for a meaningful trial. The following year, Pardi, Karikó, and Weissman went on to successfully use m1Ψ-containing mRNA encoding viral surface antigens to HIV, Zika, and influenza to elicit potent immune responses in mice and non-human primates (Pardi et al., 2018a). In an attempt to raise antibodies to a conserved region

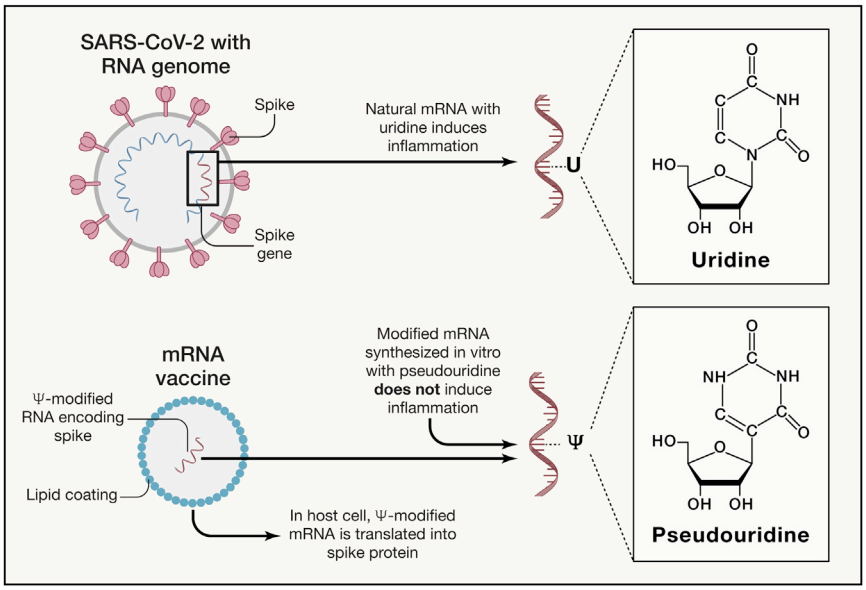

Figure 1. The design of a pseudouridine-modified mRNA vaccine for COVID-19

Schematic of the SARS-CoV-2 virus with the viral genomic RNA shown within. The receptor-binding domain of the spike protein on the surface of the virus interacts with the ACE2 receptor on the host cell, mediating viral infection. While natural messenger RNA administered via injection generally causes activation of immune system receptor proteins and resulting inflammation, *in vitro*-synthesized RNA incorporating pseudouridine (Ψ) avoids induction of inflammation. Shown are structures of uridine and its Ψ isomer. Uracil is attached to the pentose sugar by a C5-C bond in Ψ, instead of an N1-C glycosidic bond found in uridine. Nonetheless, Ψ retains the ability to pair with adenosine, explaining the capacity of the modified-mRNA to be translated. Once encapsulated in a lipid nanoparticle and injected into a host, the Ψ-mRNA is efficiently translated into protein, which serves as an antigen to elicit an immune response.

of the stalk portion of the influenza surface haemagglutinin, they were able to achieve robust antigen-specific vaccination with modified mRNA in mice, rabbits, and ferrets (Pardi et al., 2018b). In sum, this important series of investigations established the potential utility of mRNA as a therapeutic platform, including for vaccines against viral diseases.

Just as all of this progress in mRNA vaccine platforms had come into place, in January of 2020 Chinese scientists made public the sequence of the coronavirus, SARS-CoV-2, responsible for the alarming number of infections spreading worldwide. Remarkably, promising mRNA vaccine candidates were able to move from proof-of-concept to FDA Emergency Use Authorization in just 1 year. How was this possible? Both Moderna and BioNTech acted immediately to adapt the RNA technology already in house to design a vaccine based on modified mRNAs encoding the virus's spike protein, as shown in Figure 1. Moderna, which was a small company that had not yet had a drug on the market, sought and received funding from US government sources. Simultaneously, BioNTech closed a deal with Pfizer to fund development, clinical trials, and manufacturing of a COVID-19 vaccine. BioNTech and Pfizer optimized a modified mRNA-lipid nano particle vaccine against the SARS-CoV-2 spike protein that triggered strong production of neutralizing antibodies. Employing good manufacturing practice standards of production, this vaccine candidate was rapidly moved through clinical trials. The critical paper reporting the safety and efficacy of a m1Ψ-containing mRNA encoding the spike protein as a vaccine against SARS-CoV-2 was published in December of 2020 at the height of the pandemic. The results of this phase 3 trial with 43,548 people were a resounding success. Papers reporting the COVID-19 vaccine were authored by teams of researchers at both the University of Pennsylvania and BioNTech (Polack et al., 2020; Sahin et al., 2020).

Although there are many heroes in this story, Karikó and Weissman led the way to rapid generation of COVID-19 vaccines by creating a safe and effective platform based on modified mRNAs for producing therapeutic proteins. This technology has revolutionized vaccinology. Under FDA Emergency Use Authorization, the modified mRNA vaccines against SARS-CoV-2 from Moderna and Pfizer/BioNTech are saving the lives of countless numbers of people worldwide and are a major factor in quelling the COVID-19 pandemic. Looking to the future, there will be other pandemics. It is not a question of if but when other highly transmissible coronaviruses, deadly influenza viruses, or as yet unknown pathogens appear on the horizon. Fortunately, the modified mRNA vaccine strategy is highly versatile because target antigen sequences can be easily and rapidly tailored to match newly emerging viral, parasitic, or bacterial pathogens. The discovery and implementation of mRNA vaccines will continue to have an enormous impact on global health.

DECLARATION OF INTERESTS

L.S. serves on the Board of Directors of Pacific Biosciences, Inc. and was a cofounder of Anacor Pharmaceuticals, Inc. and Boragen, LLC. R.L. is on the Scientific Advisory Board of TenNor Therapeutics, Limited.

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