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mRNA Vaccines in the COVID-19 Pandemic and Beyond

Michael J. Hogan[1] and Norbert Pardi[2]

[1]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA; email: hoganm@chop.edu

[2]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; email: pnorbert@pennmedicine.upenn.edu

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), emerged in China in December 2019 and quickly spread around the globe, killing more than 4 million people and causing a severe economic crisis. This extraordinary situation prompted entities in government, industry, and academia to work together at unprecedented speed to develop safe and effective vaccines. Indeed, vaccines of multiple types have been generated in record time, and many have been evaluated in clinical trials. Of these, messenger RNA (mRNA) vaccines have emerged as lead candidates due to their speed of development and high degree of safety and efficacy. To date, two mRNA vaccines have received approval for human use, providing proof of the feasibility of this next-generation vaccine modality. This review gives a detailed overview about the types of mRNA vaccines developed for SARS-CoV-2, discusses and compares preclinical and clinical data, gives a mechanistic overview about immune responses generated by mRNA vaccination, and speculates on the challenges and promising future of this emergent vaccine platform.

INTRODUCTION

Safe and effective vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will be crucial for ending the ongoing serious pandemic of coronavirus disease 2019 (COVID-19). Due to tight cooperation among governments, regulatory agencies, and manufacturers, vaccine development proceeded with historically unprecedented speed (1). Several vaccines have already received approval for human use, and many more are in late-stage clinical development (2).

Messenger RNA (mRNA) vaccines represent a new vaccine class that has proven to be highly effective in preclinical and clinical studies against infectious diseases (3). One of the most critical advantages of this vaccine type over traditional platforms is that its synthetic nature and sequence-independent manufacturing allow extremely fast and flexible vaccine design and production (4). Due to these benefits, Moderna, Inc. designed and produced a good manufacturing practice (GMP)-quality SARS-CoV-2 mRNA vaccine (mRNA-1273) for human trials only 42 days after obtaining the nucleotide sequence of the target antigen. This feature of mRNA vaccines makes them particularly suitable for tackling rapidly emerging outbreaks. A second advantage, besides the ease of design and production, is that the SARS-CoV-2 mRNA vaccines generated by Moderna and Pfizer/BioNTech have shown very high efficacy (~90% at ≤6 months of follow-up) in phase III clinical trials and in the general population (5–8). Both vaccines have received widespread approval for human use, and administration of these regimens started in December 2020. Since then, the immunization of hundreds of millions of people with these vaccines has significantly contributed to the mitigation of COVID-19 morbidity and mortality in countries with robust vaccination programs. This clear and rapid success has thrust mRNA vaccines into the spotlight in both the scientific community and the general public.

At this critical juncture, we review the SARS-CoV-2 mRNA vaccine field, discussing preclinical and clinical data, mechanisms of immune activation (known and unknown), and perspectives on the bright future ahead for this revolutionary vaccine modality.

SARS-COV-2 MRNA VACCINE PLATFORMS AND ANTIGENS

Immune responses against several SARS-CoV-2 antigens could potentially contribute to protection from infection or viral clearance. The most widely used vaccine target is the spike (S) surface glycoprotein, which mediates viral attachment and entry via the cellular receptor angiotensin-converting enzyme 2 (ACE-2). The S protein has two subunits: S1, which contains the receptor binding domain (RBD) and multiple neutralizing epitopes; and S2, which is responsible for viral fusion with the host cell membrane (9). mRNA vaccine companies have designed and produced various versions of S-encoding mRNAs (**Table 1**), including the wild-type (WT) full-length S; a trimerized, soluble version of RBD; and, most importantly, a membrane-bound full-length construct named S-2P. This latter construct is stabilized in the prefusion conformation by proline substitutions in the S2 subunit (K986P and V987P) and is believed to induce superior neutralizing antibody responses compared to its WT counterpart, as previously observed for the S protein of Middle East respiratory syndrome coronavirus (MERS-CoV) (10). Both currently approved SARS-CoV-2 mRNA vaccines (Pfizer/BioNTech's and Moderna's) utilize the S-2P construct.

There are two major types of mRNA vaccines: those that use nonreplicating mRNA that simply encodes the target antigen, and those that use self-amplifying mRNA, which encodes both the antigen and viral genome replication proteins that replicate the mRNA within transfected cells. The former group can be further divided into unmodified and nucleoside-modified mRNA. The pros and cons of these modalities were extensively discussed elsewhere (3). All clinically advanced SARS-CoV-2 mRNA vaccine candidates (**Table 1**) use lipid nanoparticles (LNPs) to encapsulate the mRNA cargo (11). As of this writing (Sep. 30, 2021), the Pfizer/BioNTech (BNT162b2),

Table 1 Companies involved in SARS-CoV-2 mRNA vaccine development: vaccine types, vaccine administration, and status of development

Company	Name of vaccine candidate: immunogen, route of administration	mRNA dose (μg)	Development phase
Pfizer/BioNTech (US FDA approved)	BNT162b2: mod S-2P, IM	30	III (NCT04368728, 5)
	BNT162b1: mod RBD, IM	1–100	II (NCT04368728, 15, 16)
	BNT162a1: unmod RBD, IM	n/a	I (NCT04380701)
	BNT162c2: SAM S-2P, IM	n/a	I (NCT04380701)
Moderna (US FDA EUA)	mRNA-1273: mod S-2P, IM	100	III (NCT04470427, 6, 95)
CureVac	CVnCoV: unmod S-2P, IM	12	IIb/III (NCT04652102, 13)
Academy of Military Medical Science, Walvax Biotechnology, Suzhou Abogen Biosciences	ARCoV: mod RBD, IM	15	III (NCT04847102)
Translate Bio/Sanofi	MRT5500: unmod S-2P/GSAS, IM	15–135	I/II (NCT04798027, 42)
Arcturus	ARCT-021: SAM WT S, IM	5 and 7.5	II (NCT04480957)
Imperial College London	LNP-nCoVsaRNA: SAM S-2P, IM	0.1–10	I/II (ISRCTN17072692)
Daiichi Sankyo Co., Ltd	DS5670a: n/a, IM	10–100	I/II (NCT04821674)
Elixirgen Therapeutics, Inc	EXG-5003: SAM RBD, ID	n/a	I/II (NCT04863131)
GlaxoSmithKline	CoV2 SAM (LNP): SAM S, IM	1	I (NCT04758962)
Providence Therapeutics	PTX-COVID19-B: n/a, IM	16–100	I (NCT04765436) and II
SENAI CIMATEC	HDT-301: SAM S, IM	1–25	I (NCT04844268)
Chulalongkorn University	ChulaCov19: mod WT S, IM	10–50	I (NCT04566276)
MRC/UVRI, LSHTM Uganda Research Unit	LNP-nCOV saRNA-02: SAM S, n/a	5	I (NCT04934111)

Abbreviations: EUA, emergency use authorization; ID, intradermal; IM, intramuscular; LSHTM, London School of Hygiene & Tropical Medicine; mod, nucleoside-modified; MRC/UVRI, Medical Research Council/Uganda Virus Research Institute; n/a, not applicable; RBD, receptor binding domain; S, spike; SAM, self-amplifying mRNA; unmod, unmodified; WT, wild type.

Moderna (mRNA-1273) and CureVac (CVnCoV) SARS-CoV-2 vaccines had reported data from phase III trials (5, 6, 8, 12, 13). BNT162b2 and mRNA-1273 received approval for mass vaccination beginning in late 2020 via the US Food and Drug Administration (FDA) Emergency Use Authorization, and BNT162b2 has recently received full approval in the United States (14). Both vaccines use nucleoside-modified (1-methylpseudouridine-containing) S-2P mRNA formulated into LNPs. While Moderna is building its portfolio solely on the nucleoside-modified mRNA-LNP platform, BioNTech developed versions of all three major RNA-based platforms (nucleoside-modified and unmodified nonreplicating mRNA-LNP and self-amplifying mRNA-LNP, described below). BioNTech partnered with Pfizer to perform clinical studies for a trimerized nucleoside-modified RBD (BNT162b1) vaccine (15, 16), a nucleoside-modified full-length prefusion-stabilized S-2P construct (BNT162b2) (5), an unmodified mRNA-LNP (BNT162a1) vaccine, and a self-amplifying mRNA-LNP (BNT162c2) vaccine (NCT04380701). In a head-to-head phase I comparison, Pfizer/BioNTech found that BNT162b2 stimulated similar antibody responses with a lower frequency and intensity of adverse events compared to the RBD construct BNT162b1, leading to the advancement of BNT162b2 to phase II/III testing (17). Results for the other two Pfizer/BioNTech vaccines have not been published to date. CureVac AG and Sanofi/Translate Bio utilize full-length S-2P and S-2P/GSAS sequences, respectively, in their unmodified mRNA-LNP platforms. In the latter vaccine, Sanofi/Translate Bio's S-encoding mRNA also features an ablated furin cleavage site ($RRAR_{682\text{-}685}$GSAS). Finally, Arcturus Therapeutics and a research group at Imperial College London are developing self-amplifying mRNA-LNP-based

vaccines that encode the WT S and S-2P proteins, respectively. Several other companies have recently started clinical studies with various types of SARS-CoV-2 mRNA vaccines (**Table 1**).

CLINICAL SARS-COV-2 MRNA VACCINES

Development of SARS-CoV-2 mRNA vaccines has moved with unprecedented speed, and publication of preclinical and clinical data began several months after the genetic information of SARS-CoV-2 became publicly available. In this section, we review the preclinical and clinical data for SARS-CoV-2 mRNA vaccines that are clinically approved or currently under clinical investigation.

Moderna Vaccine

In January 2020, Moderna teamed up with the Vaccine Research Center of the National Institutes of Health and developed a GMP-grade batch of nucleoside-modified mRNA-LNP vaccine, mRNA-1273 (also known by the generic name elasomeran or the brand name Spikevax), encoding SARS-CoV-2 S-2P in just over a month. The vaccine was evaluated in animals in parallel with phase I clinical testing (18, 19). In mice, it induced Th1-biased S-specific $CD4^+$ T cells [desirable for antiviral immunity and avoiding concerns about Th2-related vaccine-enhanced disease (20)], $CD8^+$ T cells, and potent neutralizing antibody responses, and a single immunization with only 1 μg mRNA-LNP was sufficient to induce sterilizing protection from viral replication in the lungs (18). In nonhuman primates, dose-dependent S-specific Th1-biased $CD4^+$ T cell responses, T follicular helper cell responses, and neutralizing antibody responses were observed after two immunizations with 10 μg or 100 μg of S-2P mRNA-LNP (19). Interestingly, S-specific $CD8^+$ T cells were not strongly detected in blood. Two immunizations with the 100 μg dose induced a very high level of protection from viral replication in both the bronchoalveolar lavage fluid and nasal swabs.

Moderna started a phase I clinical trial with 45 healthy individuals in mid-March 2020 (21). Participants were intramuscularly immunized twice, 4 weeks apart, with 25 μg, 100 μg, or 250 μg of vaccine. Vaccine-elicited adverse events (fever, chills, headache, myalgia, fatigue, etc.) were dose-dependent and were significantly stronger after administration of the second dose. The vaccine induced robust, dose-dependent humoral immune responses as measured by S-specific IgG titers and virus neutralization assay, with a strong boost effect from the second immunization. This phase I trial was later expanded to include 40 older people divided into two groups (aged 56–70 years and >71 years) (22). mRNA-1273 induced mainly mild and moderate adverse events in older people that were usually stronger after the second vaccine dose, and neutralizing antibodies were roughly similar to those elicited in vaccinees 18–55 years old. As in nonhuman primates, S-specific $CD4^+$ T cell responses were strong and Th1-biased, and $CD8^+$ T cell responses were low and variable, as measured by intracellular cytokine staining. It is not fully clear whether the $CD8^+$ T cell response is lower in people immunized with the Moderna vaccine compared to Pfizer/BioNTech, since a separate study using a distinct assay (activation induced marker expression) was able to detect similar, fairly strong $CD8^+$ T cell responses in people immunized with both vaccines (23).

Based on these initial safety and immunogenicity results, the company started a phase III observer-blinded clinical trial with 30,420 participants randomized 1:1 to receive placebo or 100 μg of vaccine (6). Participants were 18–65 years old and received two immunizations 4 weeks apart of the vaccine or placebo. Adverse vaccine reactions were more common after the second dose and were self-limiting. Local adverse events after dose 2 were generally mild to moderate, while systemic adverse events after dose 2 were more frequently reported as moderate (38.1%) to

severe (15.8%). The vaccine demonstrated 94.1% efficacy at preventing COVID-19 illnesses and complete protection from severe COVID-19 during a median follow-up time of 64 days, and Moderna later reported that efficacy remained high (>90% against all COVID-19 and >95% against severe COVID-19) after a median of 6 months of follow-up (24). Recent publications demonstrated that mRNA-1273-induced neutralizing antibody titers against multiple SARS-CoV-2 variants decreased gradually over 180 days after the second vaccine dose to roughly 20% of the peak response (25, 26). Evaluation of immune responses will be continued for 2 years to determine the long-term durability of immunity. Based on the demonstrated safety and impressive efficacy, mRNA-1273 received authorization for emergency use in the United States on Dec. 18, 2020. Shortly after, many countries around the globe started to use this vaccine, and hundreds of millions of doses have been administered to people in 72 countries as of September 2021 (27).

Pfizer/BioNTech Vaccine

Like Moderna's, Pfizer/BioNTech's most clinically advanced vaccine, BNT162b2 (also known by the generic name tozinameran or the brand name Comirnaty), is based on nucleoside-modified S-2P mRNA-LNPs (5, 17, 28–31). BNT162b2 induced potent cellular and humoral immune responses in mice after a single immunization with 0.2–5 μg of mRNA and similarly robust, highly protective immune responses in rhesus macaques after two immunizations with 30 μg or 100 μg doses (31).

In phase I clinical testing, study participants received two immunizations (3 weeks apart) with 1, 10, 20, or 30 μg of BNT162b2. The vaccine was well-tolerated and adverse events were dose dependent. A single immunization induced low levels of neutralizing antibodies that were significantly boosted by 7 days after the second dose, administered on day 21. Small differences in neutralizing antibody titers between participants who received 10, 20, or 30 μg vaccine were observed. All vaccine doses induced Th1-biased $CD4^+$ and $CD8^+$ T cell responses (30). Importantly, BNT162b2 proved to be safe and immunogenic in older adults (65–85 years of age), inducing dose-dependent neutralizing antibody titers that were comparable to or higher than those measured in SARS-CoV-2 convalescent sera (17).

BNT162b2 is undergoing evaluation in a large multinational observer-blinded phase III clinical study (NCT04368728; 5). A total of 43,548 volunteers (16 years or older) were assigned to vaccine or placebo in a 1:1 ratio. Study participants received two immunizations with 30 μg BNT162b2, 3 weeks apart. Adverse reactions were generally mild to moderate and self limiting; the most common severe adverse event, fatigue, occurred in 3.8% of participants. The incidence of serious (grade 4) adverse events was low and was similar in both groups of participants (0.6% in the vaccine group and 0.5% in the placebo group). An impressive efficacy of 95% was measured during a median follow-up of 2 months: 8 cases of COVID-19 with onset at least 7 days after administration of the second dose were found in the vaccine group, and 162 cases were found in the placebo group.

A follow-up publication evaluated safety and efficacy of BNT162b2 6 months after vaccination. The vaccine continued to be safe, and the efficacy remained high: 91.3% efficacy overall among vaccinees without evidence of previous SARS-CoV-2 infection, and 83.7% efficacy at ≥4 months after dose 2 (until no more than 7.5 months after dose 2) (8). In this analysis, 2,264 participants aged 12–15 years were also included. Interestingly, vaccine efficacy was 100% in South Africa, where the variant of concern Beta was predominant, although the sample size in this population was somewhat limited, and other variants of concern were not analyzed here. Importantly, the efficacy of at least one dose of BNT162b2 against severe COVID-19 remained extremely high (96.7%) through 6 months of follow-up. Based on these safety, immunogenicity, and efficacy

results, BNT162b2 has received approval for human use in 100 countries, and at least 2.1 billion doses have been prepurchased for delivery by the end of 2021 (32). In early studies, both the neutralizing antibody responses (33) and efficacy in preventing symptomatic COVID-19 within 2 months of follow-up (5, 6) were indistinguishable between the Moderna and Pfizer/BioNTech vaccines, made using similar nucleoside-modified mRNA-LNP technologies. Interestingly, a recent publication demonstrated that mRNA-1273 elicited 2.7-fold higher RBD-specific immunoglobulin titers than BNT162b2 in Belgian healthcare workers (34), and an analysis by the US Centers for Diseases Control and Prevention (CDC) found that vaccine efficacy against COVID-19 hospitalization was slightly higher after vaccination with mRNA-1273 compared to BNT162b2 (35); however, these comparisons are not definitive given the fact that the populations are not randomized and there may be differences in risk factors for COVID-19.

CureVac Vaccine

CureVac's unmodified, sequence-engineered (GC-rich) (36) S-2P mRNA-LNP vaccine, CVnCoV, was evaluated preclinically in rodents and nonhuman primates (37, 38). In mice, the vaccine induced robust $CD4^+$ and $CD8^+$ T cell responses and neutralizing antibody responses after two immunizations with 2 μg mRNA-LNP. In Syrian golden hamsters, a high level of protection from SARS-CoV-2 replication in the lungs was achieved by two immunizations of 10 μg, despite significantly lower neutralizing antibody responses compared to mice (37). CVnCoV was evaluated in nonhuman primates as well, and two immunizations with only 8 μg vaccine induced strong cellular and humoral immune responses and a high level of protection from viral replication (38).

Clinical evaluation of CVnCoV started in June 2020 in a phase I safety and immunogenicity study (NCT04449276). Two immunizations with multiple vaccine doses (2, 4, 6, 8, 12, 16, and 20 μg) were performed in 250 healthy individuals (39). CVnCoV-induced adverse events were dose dependent; the safety profile of the vaccine was generally acceptable, but ~35% of participants in the 12 μg group experienced severe systemic adverse events after the second vaccination, a higher percentage than the modified mRNA vaccines from Moderna and Pfizer/BioNTech (5, 6). All vaccine doses induced antigen-specific humoral immune responses after two immunizations, and higher doses elicited stronger responses. Interestingly, there appeared to be significant variability in antibody responses to 12 μg CVnCoV, though the sample size was small. CureVac has started a phase IIb/III randomized placebo-controlled blinded safety and efficacy trial (NCT04652102) with 39,680 participants (vaccine and placebo groups in a 1:1 ratio) in ten countries using two immunizations 28 days apart with the 12 μg dose (13). Primary efficacy analysis included symptomatic COVID-19 more than 14 days after administration of the second dose. Primary vaccine efficacy was 48.2%, while efficacy against moderate to severe COVID-19 was 70.7%, significantly lower than that of the Moderna and Pfizer/BioNTech vaccines. It is possible that some of the difference in primary vaccine efficacy is due to the predominance of variants of concern in the CVnCoV trial. Solicited adverse events (mostly systemic) were more common in the vaccine group (~27%) compared to the placebo group (~3.1%). Overall, CVnCoV has an acceptable safety profile but its efficacy is lower than that of the Moderna and Pfizer/BioNTech COVID-19 mRNA vaccines.

Additional SARS-CoV-2 mRNA Vaccines

Several other mRNA-based COVID-19 vaccine candidates have entered clinical trials (**Table 1**), but no or very limited clinical data have been released from most of these ongoing studies. A

modified mRNA-LNP vaccine encoding the S RBD (ARCoV) is being developed by the Academy of Military Medical Sciences (Beijing), Walvax Biotechnology, and Suzhou Abogen Biosciences. This vaccine induced potent neutralizing antibody responses, $CD4^+$ and $CD8^+$ T cell responses, and protection from SARS-CoV-2 infection in mice after prime-boost immunizations with 2 and 10 μg doses (40). Two immunizations with 100 and 1,000 μg doses induced robust neutralizing antibody responses in cynomolgus macaques. The vaccine is being evaluated in a randomized double-blind placebo-controlled phase III clinical trial with ~28,000 study subjects including ~7,000 individuals who are older than 60 years of age (NCT04847102). Participants received two immunizations with 15 μg mRNA-LNPs, 4 weeks apart, and safety and efficacy are being evaluated.

Sanofi/Translate Bio has tested the immunogenicity of its MRT5500 unmodified S-2P/GSAS mRNA-LNP vaccine in preclinical models (41). Two immunizations (3 weeks apart) with 1–10 μg doses induced robust antigen-specific IgG and neutralizing antibody responses in mice, and 15–135 μg doses induced both strong cellular and humoral immunity in nonhuman primates. Evaluation of MRT5500 entered phase I/II testing in March 2021 (42).

Self-amplifying mRNA-LNP vaccines have also undergone preclinical and early-stage clinical testing for SARS-CoV-2. Scientists from the Imperial College London found that two immunizations with even very small amounts (10 ng) of S-2P self-amplifying mRNA-LNP induced robust cellular and humoral immune responses in mice (43). This vaccine (LNP-nCoVsaRNA) is currently being evaluated in a phase I/II clinical trial (ISRCTN17072692).

Similarly, Arcturus's ARCT-021 clinical vaccine candidate utilizes a self-amplifying full-length WT S-encoding mRNA with proprietary LNPs. It induced robust, dose-dependent cellular and humoral immune responses in mice, and a high level of protection was obtained with a single immunization of 2 μg (44). It was also demonstrated that ARCT-021 induced protective immune responses in B cell–depleted but not $CD8^+$ T cell–depleted mice, suggesting that robust $CD8^+$ T cell responses may be sufficient to achieve protection from viral replication, at least in the short term (45). Two immunizations with 5 μg or a single immunization with 20 μg also induced a high level of protection from viral replication in monkeys. ARCT-021 was then tested in a phase I/II safety and immunogenicity trial. Study participants received a single dose of 5 μg or 7.5 μg or two doses of 5 μg vaccine or placebo. The vaccine was well-tolerated and induced $CD4^+$ and $CD8^+$ T cell responses but, surprisingly, low neutralizing antibody responses. The company has started a phase II clinical trial with ~600 participants (45).

Several other small phase I and phase I/II studies utilizing self-amplifying or nonreplicating mRNA vaccine platforms have recently been initiated. To our best knowledge, no published data are available on the safety and immunogenicity of these vaccine candidates. Publicly available data about these clinical trials are summarized in **Table 1**.

MECHANISM OF ACTION

The two COVID-19 mRNA vaccines that have been proven efficacious to date (i.e., Moderna's and Pfizer/BioNTech's) share the same technological approach: Both express the SARS-CoV-2 S-2P protein via nucleoside-modified mRNA encapsulated in LNPs. In this section, we describe what is known and unknown regarding the mechanisms by which this vaccine platform elicits protective immune responses, and we highlight areas in need of further research.

Correlates of Protection from COVID-19

As of Sep. 30, 2021, a preponderance of evidence suggests that total S-specific immunoglobulin G (IgG), RBD-specific IgG, and SARS-CoV-2 neutralizing antibodies are effective correlates of protection for all COVID-19 vaccines, including those using mRNA (46, 47). To date, the most

rigorous correlates analysis available for one of the COVID-19 mRNA vaccines was performed by Gilbert et al. as part of Moderna's phase III efficacy trial (47). The authors found that the risk of COVID-19 in study participants decreased by 58% for each 10-fold increase in day 57 SARS-CoV-2 pseudovirus-neutralizing antibodies [expressed as a 50% inhibitory dilution (ID_{50}) calibrated to the World Health Organization standard], with a titer of 100 associated with a vaccine efficacy of 90.7% and a titer of 1,000 associated with 96.1% efficacy through 100 days of follow-up. Somewhat weaker associations were observed for S- and RBD-specific IgG. One caveat of this study is that the analysis was performed prior to the dominance of SARS-CoV-2 variants of concern.

Experimentation in animal models supports a mechanistic basis for SARS-CoV-2 protection by S-specific IgG and neutralizing antibodies. Several studies show that S-specific antibodies or convalescent IgG are sufficient to confer a high level of protection against COVID-19 and SARS-CoV-2 replication in animal models (48–50). McMahan et al. examined the protection conferred to rhesus macaques by antibodies by performing an adoptive transfer of IgG from SARS-CoV-2 convalescent monkeys to naïve monkeys (49). They found that a pseudovirus neutralization ID_{50} titer of >46 was sufficient to grant sterilizing immunity, and lower titers were associated with detectable but reduced viral replication. In the case of humans vaccinated with COVID-19 mRNA vaccines, neutralization titers are much higher (approximately 5–20× higher, depending on the assay) than in this study, so we speculate that these antibody responses are sufficient to afford protection. Another important finding in the McMahan et al. study was that $CD8^+$ T cells were also capable of contributing to protection from reinfection in SARS-CoV-2 convalescent macaques where neutralizing antibodies were waning to around the threshold of protection (49). Interestingly, in another study, mice immunized with S-encoding modified mRNA-LNP vaccines developed strong $CD8^+$ T cell responses in the lungs, suggesting that these cells might be primed to home to sites of infection to control SARS-CoV-2 replication (51). Both the Pfizer/BioNTech and Moderna vaccines induce some level of $CD8^+$ T cell and $CD4^+$ T cell response (23, 30), so it is possible that these cells also contribute to protection in humans, although this has not been examined in any rigorous way. Since the data for long-term protection is strongest for antibodies, we focus in the following subsections on how the nucleoside-modified mRNA-LNP vaccine platform stimulates potent neutralizing antibody responses.

Potent Induction of T Follicular Helper Cells, Germinal Centers, and Neutralizing Antibodies

The first report of a nucleoside-modified mRNA-LNP vaccine was published in 2017 (52), and over the ensuing 4 years it has become apparent that this vaccine platform is regularly associated with potent and very durable neutralizing antibody responses in preclinical models, after either a single immunization (52, 53) or two immunizations (54–56). It therefore is no surprise that the modified mRNA-LNP vaccines for SARS-CoV-2 share this hallmark of potent neutralizing antibody responses. This was evidenced both for the Moderna and Pfizer/BioNTech clinical vaccines after two injections in humans (15, 17, 21, 22) and for preclinical SARS-CoV-2 vaccines after a single injection in mice (40, 51).

Potent and long-lived neutralizing antibody responses originate in germinal centers (GCs), the sites within lymph nodes where GC B cells undergo somatic hypermutation, receive help from T follicular helper (Tfh) cells, and ultimately produce high-affinity antibodies. This process is necessary for the generation of class-switched antibodies (e.g., IgG or IgA), memory B cells, and, perhaps most importantly, long-lived plasma cells that can secrete protective antibodies for up to the lifetime of the host. We and others have measured the Tfh and GC B cell responses generated by earlier nucleoside-modified mRNA-LNP vaccines and found that they are remarkably strong

compared to other vaccine types in both mice and rhesus macaques (53, 57). In 2020, Lederer et al. examined the Tfh and GC B cell response to SARS-CoV-2 modified mRNA-LNP vaccines in mice and showed that the trend holds true here as well (58). After vaccination with SARS-CoV-2 spike RBD mRNA-LNP, large GCs were visualized in draining lymph nodes after 7 days. In contrast, when mice were immunized with RBD as a protein mixed with Addavax (an MF59-like adjuvant), GCs were minimal or nonexistent. Accordingly, the authors detected large expansions in the number and frequency of Tfh and GC B cells (total and antigen-specific) in draining lymph nodes after immunization with RBD mRNA-LNP but not protein. Strong GC responses were associated with high numbers of RBD-specific bone marrow–resident plasma cells and memory B cells. These findings offer a mechanistic basis (elaborated in 59) for the potent neutralizing antibody response obtained in mice after a single injection with this vaccine (51, 58) and in humans given the Moderna and Pfizer/BioNTech vaccines (15, 17, 21, 22).

Exactly how nucleoside-modified mRNA-LNP vaccines elicit such strong GC responses remains unclear; however, there is evidence from animal studies that the response is promoted by both nucleoside modification and the LNP carrier, which each contribute in overlapping ways to efficient translation of the encoded antigen and a favorable cytokine milieu that together support the GC reaction. This model is represented graphically in **Figure 1** and is described in the following two subsections.

Nucleoside Modification and mRNA Preparation

Prior to 2005, the primary issues with using mRNA for therapeutic purposes were insufficient expression and excessive innate inflammatory reactions in vivo. A series of landmark studies by Karikó, Weissman, and colleagues demonstrated that the two problems were linked (60). A key discovery was that, while synthetic mRNA was highly inflammatory when used to transfect dendritic cells, transfer RNA (tRNA) was noninflammatory. Karikó et al. reasoned, correctly, that the difference in sensing between the two types of RNA could be due to tRNA's many chemically modified nucleosides. Indeed, unmodified mRNA is sensed by a variety of Toll-like receptors (TLR7, TLR8, TLR3) (60) and other intracellular RNA sensors [e.g., protein kinase RNA-activated (PKR) and 2′-5′-oligoadenylate synthetase (OAS)] (61, 62), leading to a signaling cascade that significantly dampens protein expression (3). In contrast, incorporation of modified nucleosides such as pseudouridine was shown to reduce or abrogate this innate sensing (60–62), allowing a high level of protein expression in vivo (63, 64). In 2015, Andries et al. demonstrated that another modified nucleoside, N1-methylpseudouridine, outperformed pseudouridine, with less sensing by TLR3 and greater expression in vivo (65); this nucleoside would go on to become the standard in the field, used by both Moderna and Pfizer/BioNTech in their COVID-19 vaccines as well as in numerous preclinical studies by our group and others.

Nucleoside modification has benefits beyond merely increasing protein expression. In 2018, we showed that an mRNA-LNP vaccine encoding influenza virus hemagglutinin was able to elicit strong GC reactions and Tfh cells only when the mRNA was N1-methylpseudouridine-modified but not when unmodified (53). The mechanism of this effect is not understood, but possible contributors include (*a*) increased and prolonged antigen availability overall (53, 66), (*b*) increased antigen expression specifically in professional antigen-presenting cells (which may preferentially express RNA sensors and thus be more sensitive to unmodified mRNA), and (*c*) a more favorable cytokine milieu (67).

Besides nucleoside modification, other aspects of mRNA preparation can potentially affect the immune response. Purification of synthetic mRNA to remove double-stranded RNA (dsRNA) contaminants, e.g., by high-performance liquid chromatography (68) or cellulose adsorption (69),

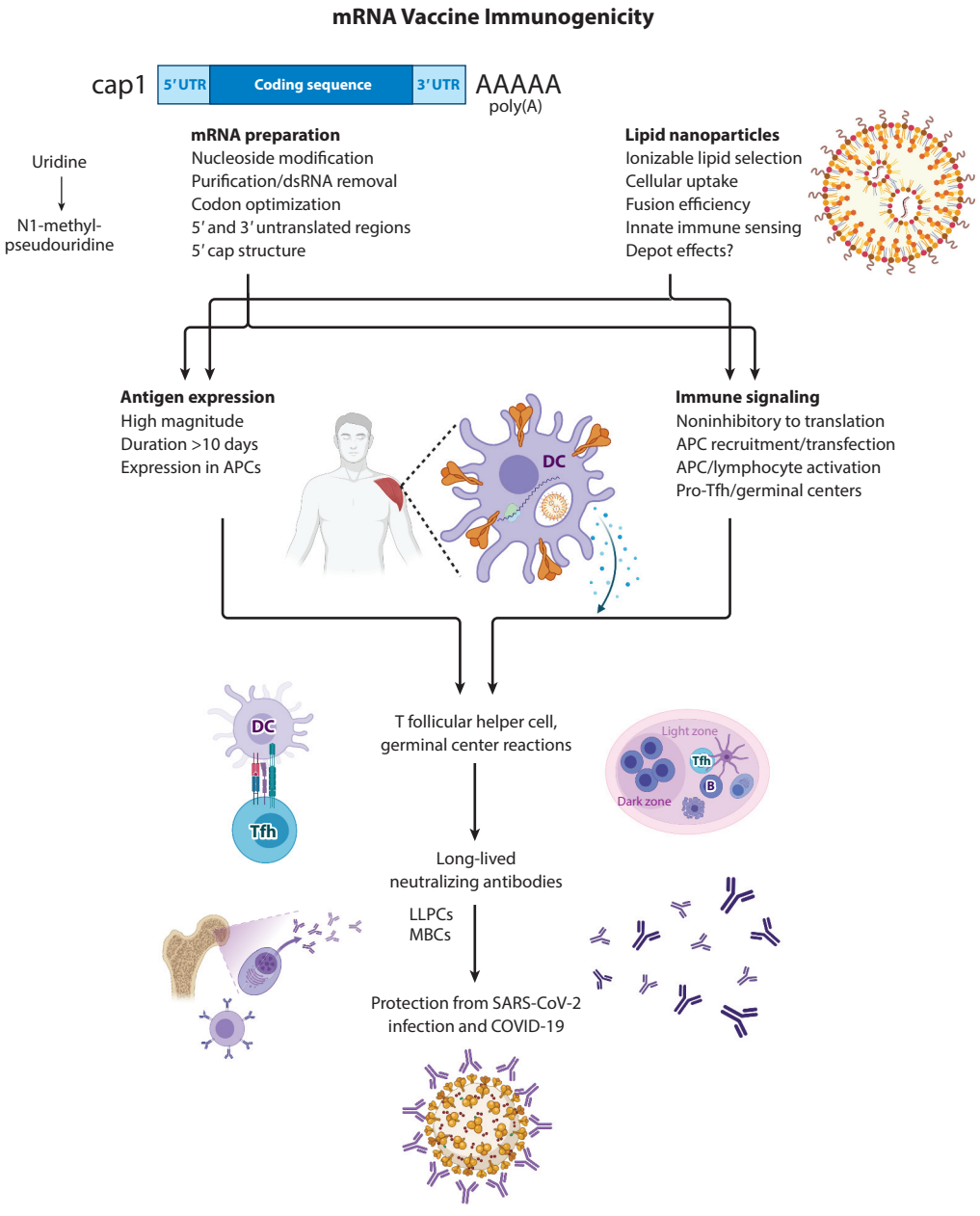

Figure 1

Mechanisms of mRNA-LNP vaccine immunogenicity. Depicted are the key, often overlapping aspects of mRNA-LNP vaccines leading to potent neutralizing antibody responses, a major contributor to protective immunity. $CD8^+$ T cell immunity is not shown for simplicity. Abbreviations: APC, antigen-presenting cell; COVID-19, coronavirus disease 2019; DC, dendritic cell; ds, double-stranded; LLPC, long-lived plasma cell; LNP, lipid nanoparticle; MBC, memory B cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Tfh, T follicular helper; UTR, untranslated region. Figure adapted from images created with BioRender.com.

was shown to be critical for in vivo expression of unmodified mRNA, but the effect is much less pronounced for modified mRNA, as it is sensed much less by intracellular dsRNA sensors. Codon optimization and the RNA 5′ and 3′ untranslated regions have also been shown to impact protein expression and immunogenicity (36).

The Double Duty of Lipid Nanoparticles: Carrier and Adjuvant

COVID-19 mRNA vaccines utilize ionizable LNPs to protect the mRNA from degradation by RNases and deliver it to the cytosol of cells in vivo so that it may be translated into protein. Ionizable LNPs were originally designed to deliver siRNA to the liver but were repurposed to deliver mRNA starting in 2015 and were found to be extremely efficient carriers (70) and potent inducers of adaptive immunity (52). Ionizable LNPs in the Moderna and Pfizer/BioNTech vaccines are composed of four components: an ionizable cationic lipid with a pKa between 6 and 7, cholesterol, a phosphatidylcholine, and a polyethylene glycol (PEG)-conjugated lipid. The details of ionizable LNP chemistry are described in great detail in a recent review (71). The basic principle is that the ionizable cationic lipid binds the negatively charged RNA at low pH to form an electron-dense core, while the surface of the particle reverts to neutral at physiologic pH. When the LNP is endocytosed and undergoes acidification, the surface ionizable lipid becomes positively charged and fuses with the endosomal membrane, delivering the RNA cargo to the cytosol. The ionizable lipid is therefore a critical component in determining the efficiency of mRNA delivery. A prototype ionizable lipid is Dlin-MC3-DMA (abbreviated MC3), which was used in several preclinical and clinical mRNA-LNP vaccines (54, 56). More efficient versions were developed more recently (72) and are included in the COVID-19 mRNA vaccines; Moderna uses SM-102 while Pfizer/BioNTech uses ALC-0315 (71). This advancement is almost certainly one of the factors responsible for the potency of these vaccines.

Serendipitously, ionizable LNPs or mRNA-LNP complexes can also serve as extremely effective adjuvants. Swaminathan et al. used empty ionizable LNPs to successfully adjuvant vaccines containing hepatitis B or dengue virus proteins (73, 74), and our group found that mRNA-LNP complexes encoding firefly luciferase were able to potently adjuvant an influenza hemagglutinin protein vaccine (53). It appears that the ionizable lipid is an important mediator of the adjuvant effect, since Hassett et al. tested LNPs with a variety of different ionizable cationic lipids and found that this component could modulate both the innate immune sensing [e.g., interleukin (IL)-6 secretion] and the antibody response (72).

The idea of LNP as adjuvant is little appreciated within the scientific community, yet it may be one of the most critical features driving the protective immune responses in these vaccines. How ionizable LNPs are sensed, if it all, is currently unknown. One possibility is that there is a cellular receptor, such as a TLR, that specifically recognizes the whole LNP, the ionizable lipid or other lipid components, or a lipid metabolite. There is some precedent for this in reports that cationic lipids used to make liposomes can be sensed by TLR4 (75), TLR2, and NOD-like receptor protein 3 (NLRP3) (76). Another possibility is that there is a mechanism that senses cationic membrane patches or membrane disturbances caused by fusion between the LNP and endosomal membrane, as postulated by Holm et al. (77). A final possibility is that LNPs mediate their adjuvant effect indirectly, e.g., through an antigen depot effect, delivery of mRNA to compartments where it can be sensed, by directing antigens to antigen-presenting cells, and/or by damaging cells and releasing damage-associated molecular patterns (78).

Another mysterious aspect of the LNP adjuvant effect is which innate immune signals are important to drive T and B cell responses. A few studies have provided hypothesis-generating information. Lutz et al. showed that mRNA-LNP complexes, but not mRNA alone, stimulated

systemic release of IL-6, tumor necrosis factor, MIP-1β (macrophage inflammatory protein 1β), and CXCL9 (C-X-C chemokine ligand 9) after intramuscular injection in mice (79). Ndeupen et al. showed that various chemokines and cytokines were produced locally when LNPs containing poly-cytosine (noncoding) RNA were injected into mice; these included high levels of CCL2 (C-C chemokine ligand 2), CCL3, CCL4, CCL7, CCL12, CXCL1, CXCL2, IL-1β, and IL-6 (78). In accordance with this chemokine production, this study and another in rhesus macaques identified a rapid local infiltrate of monocytes, macrophages, dendritic cells, neutrophils, and other cells following injection with empty LNPs (78) or modified mRNA-LNPs (80). It seems logical that this rich environment of chemokines and antigen-presenting cells would promote major histocompatibility complex class II presentation to $CD4^+$ T cells, potentially driving Tfh cell differentiation and GC reactions. However, clearly, much more work is needed to understand this process fully.

EFFECTIVENESS IN THE GENERAL POPULATION

Prevention of SARS-CoV-2 Infection and COVID-19

While phase III studies (5, 6) demonstrated a high level of efficacy of the Moderna and Pfizer/BioNTech mRNA-LNP vaccines, it is important to determine the level of protection outside the context of a well-controlled clinical trial and in a general population. The highest-quality early data on this question came from Israel, which organized an extremely rapid vaccination campaign starting in December 2020. In a study published by Dagan et al. (7), 596,618 individuals were vaccinated with the Pfizer/BioNTech vaccine within Israel's largest health care system, Clalit Health Services, and were matched 1:1 to demographically similar unvaccinated individuals. Rates of SARS-CoV-2 infection and COVID-19 were compared between groups. The results were striking: Starting 7 days after the second vaccine dose, there was an estimated 92% protection against documented SARS-CoV-2 infection, 94% protection against symptomatic COVID-19, 92% protection from severe COVID-19, and an 87% reduction in hospitalization due to COVID-19. There appeared to be similarly strong protection against COVID-19 mortality, although a statistical comparison was not made after the second dose as there were very low mortality case numbers. Protection from asymptomatic infection was estimated at 90%. Partial effectiveness was observed after a single dose but was significantly boosted by the second immunization. Protection from disease was high (>88%) in every subset of people stratified by sex, age, or underlying health conditions. A similar, smaller report from the United States showed comparable findings, with 90% effectiveness of the two mRNA vaccines (combined) in preventing all SARS-CoV-2 infections (81). These early studies also showed a high degree (>90%) of prevention of total SARS-CoV-2 infections, raising hopes that the vaccine would block nearly all viral transmission; however, more recent data from the summer of 2021 indicated that breakthrough infections were becoming more common for both mRNA-1273 and especially BNT162b2, likely due to the gradual waning of immune responses and/or the increasing dominance of variants of concern, in particular the Delta variant (82, 83). Nevertheless, both mRNA vaccines continued to be highly protective against severe COVID-19 outcomes such as hospitalization and intensive care unit admission.

An additional consideration for the effectiveness of COVID-19 vaccines is protection against long-term sequelae of SARS-CoV-2 infection, also known as "long COVID." While there is currently a dearth of high-quality data documenting the frequency, severity, and duration of long COVID symptoms and the protection afforded by vaccines, one case-control study monitoring self-reported symptoms showed that COVID-19 vaccines in the United Kingdom (33% Pfizer/BioNTech and 66% AstraZeneca vaccines) halved the frequency of COVID-19 symptoms

reported at ≥28 days after vaccine breakthrough infections, indicating an additional benefit of vaccination in protecting from long COVID (84).

Variants of Concern

As an RNA virus, SARS-CoV-2 naturally mutates at a high rate and selects for variants with improved fitness (transmission and replication) and/or immune escape. Over the past year, several new lineages of SARS-CoV-2 have been named variants of concern by the World Health Organization based on the presence of genetic changes that increase transmissibility, virulence, or resistance to medical interventions including vaccines. These include Alpha (B.1.1.7), first identified in the United Kingdom; Beta (B.1.351), first identified in South Africa; Gamma (P.1), first identified in Brazil; and Delta (B.1.617.2), first identified in India. Of these variants, Beta is associated with the greatest resistance to neutralizing antibodies in convalescent and vaccine sera, while Delta is associated with modestly reduced neutralization and an extremely high rate of transmission (26, 85). As of the preparation of this review in September 2021, the Delta variant has overcome all other viral lineages and become dominant in many countries around the world, potentially due to highly efficient viral entry and replication as measured in vitro (86).

The emergence of these variants of concern has raised questions regarding the breadth of immunity afforded by vaccination and the current level of effectiveness of the COVID-19 mRNA vaccines. Some studies have shown that the COVID-19 mRNA vaccines are highly effective even against the Alpha (7), Beta (8), and Delta (87) variants, while others suggest that Delta is able to escape vaccine immunity much more commonly than other strains (82, 83, 88). A possible contributor to this discrepancy is differing intervals of time that had elapsed between vaccination and exposure to the Delta virus in these studies. Due to these concerns, Moderna and Pfizer/BioNTech have initiated studies to examine the impact of booster vaccination with their original vaccines or sequence-updated versions that reflect variants of concern, with the goal of increasing the magnitude and breadth of neutralizing antibody responses. After promising results in mice (89), Moderna is launching multiple clinical trials to examine booster immunizations with mRNA vaccines encoding wild-type, Beta, Delta, wild-type and Beta, or Beta and Delta S protein sequences (90). Pfizer/BioNTech is also expected to study booster vaccines based on the Beta and Delta variants as well as wild-type S protein (results for the latter discussed below).

Safety in the General Population

While the Moderna and Pfizer/BioNTech vaccines were shown to be safe in tens of thousands of people in phase III studies (5, 6), it is necessary to further study safety during administration of vaccines to the general population in the event that there are rare serious adverse events (i.e., occurring in fewer than ~1 in 10,000 cases). In the United States, where the two vaccines were initially administered in the greatest numbers, there are multiple mechanisms by which information on adverse events is collected on an ongoing basis, as detailed below.

The Vaccine Adverse Event Reporting System (VAERS), overseen by the FDA and CDC, is a hypothesis-generating system by which adverse events can be reported after vaccination to allow investigation of potential vaccine safety issues. After initial reports of rare cases of anaphylaxis after COVID-19 mRNA vaccine administration, data obtained from VAERS between December 2020 and January 2021 were used to estimate that anaphylaxis occurred in 4.7 cases per million doses of Pfizer/BioNTech vaccine and 2.5 cases per million doses of Moderna vaccine (91). Of the 66 cases of anaphylaxis reported in this study, 95% were female, nearly all patients received epinephrine as a treatment, and no deaths were reported. The Vaccine Safety Datalink is an ongoing project to detect a predefined set of rare serious adverse events caused by vaccination by comparing their

incidence in a large, diverse, vaccinated population relative to the background incidence in the United States. This mechanism was used to further study the risk of anaphylaxis and estimated a rate of 5 cases of anaphylaxis per million doses for both mRNA-1273 and BNT162b2 as of May 29, 2021 (92). While the cause of the anaphylactic reactions is not known, the PEG-lipid in the LNP carrier has been hypothesized to be a possible trigger, as anti-PEG antibodies are commonly detectable in healthy human blood, and PEGylated liposomes have been known to elicit hypersensitivity reactions (93).

VAERS also received reports of rare cases of myocarditis and pericarditis following vaccination with the Moderna and Pfizer/BioNTech vaccines, particularly in younger individuals following the second dose, and more commonly in males (94). The Vaccine Safety Datalink was used to estimate the excess incidence of myocarditis/pericarditis as roughly 11 cases per million for individuals age 12–39 years in the week after receiving the second dose of vaccine, as of June 26, 2021 (92). Among 304 CDC-reviewed cases of myocarditis/pericarditis, acute clinical courses were generally mild and no deaths had been reported (94).

Finally, Pfizer/BioNTech has shared that the participants in their phase III trial have not experienced any serious safety concerns through 6 months of follow up (8).

CHALLENGES AND QUESTIONS

Populations Excluded from Initial Phase III Trials

Due to potentially increased risk of adverse events and ethical concerns, several populations were excluded from the initial phase III clinical trials of the Moderna and Pfizer/BioNTech vaccines (5, 6). These include people who are pregnant or breastfeeding, people who have immune-compromising conditions or who take immune-modulating medication, and adolescents and children below the ages of 16 (Pfizer/BioNTech) or 18 years (Moderna). However, regulatory agencies from the outset have authorized administration of these vaccines to all individuals of approved age. The delay in safety data for these groups may have created some level of hesitancy to seek vaccination, particularly among pregnant women (96), who are at increased risk of preterm birth and severe illness due to COVID-19 (97).

As of the preparation of this review, significant progress has been made to address the questions of safety and efficacy in these populations. Several months after authorization of their vaccine in adults, Pfizer/BioNTech reported its vaccine to be safe, highly immunogenic, and 100% efficacious in adolescents aged 12–15 years (8, 98), with emergency use now authorized in this group by the FDA. Moderna has announced similar results (99). More recently, Pfizer/BioNTech reported that a one-third (10 μg) dose of their vaccine was well tolerated in children aged 5–11 years and had similar immunogenicity compared to young adults aged 16–25 years receiving the full 30 μg vaccine, and that a 3 μg dose is under investigation in children aged 6 months to 4 years (100). Similarly, Moderna is testing their vaccine in children as young as 6 months of age in a dose-escalating and age-deescalating manner (NCT04796896).

The safety and efficacy of COVID-19 mRNA vaccines in pregnant women have now been evaluated by multiple types of studies. A small cohort study showed that S-specific IgG and side effects were similar between pregnant and nonpregnant women (101). The CDC launched the v-safe COVID-19 Vaccine Pregnancy Registry as a method to gather self-reported data on the safety of COVID-19 vaccines in pregnant women. As of Feb. 28, 2021, these data indicated no safety issues in a population of 35,691 pregnant people (102). Pfizer/BioNTech is conducting a randomized placebo-controlled phase II/III trial (NCT04754594) to examine vaccine safety and immunogenicity in thousands of pregnant women, and Moderna has indicated similar plans (6).

Medical institutions around the globe have led efforts to examine the safety and efficacy of the mRNA-based and other COVID-19 vaccines in people with various forms of immune deficiency or immune suppression (e.g. NCT04780659, NCT04828460, NCT04799808, NCT04805125, NCT04805216, NCT04806113, and NCT04769258). A very informative study recently shared by Bergman et al. examined safety and immunogenicity of the Pfizer/BioNTech vaccine in people with primary immunodeficiency, HIV infection, hematopoietic stem cell transplantation, chimeric antigen receptor T cell therapy, solid organ transplantation, or chronic lymphocytic leukemia (103). They found lower and more variable antibody titers compared to healthy controls, with the lowest rates of seroconversion among those with solid organ transplantation (43%) or chronic lymphocytic leukemia (63%). Adverse events were generally mild, but two severe/serious adverse events were observed in transplant recipients, warranting caution and a need for further study on the safety of COVID-19 mRNA vaccination in transplant recipients. Given the lower rate of seroconversion in severely immunocompromised people, the FDA and other regulatory agencies have recommended a third immunization with BNT162b2 or mRNA-1273 (not deemed a "booster immunization") in this population.

In future epidemics, it should be possible to safely expedite the testing and deployment of vaccines in many medium- or high-risk populations by altering phase I–III clinical trial designs so that these populations are (*a*) recruited early with informed consent, (*b*) automatically included in trials as soon as predetermined benchmarks of safety and potentially immunogenicity are reached in low-risk adults (104), and (*c*) given an appropriate level of medical care and monitoring.

Cost and Availability

Despite being a new technology on the market, the mRNA vaccines for COVID-19 have been mass produced at a reasonable cost. For example, by February 2021, the US government had prepurchased 300 million doses of vaccine from Pfizer/BioNTech for approximately $19.50 per dose (105). This is notably cheaper than many cutting-edge vaccines, most likely due to the exigency of the pandemic. Pfizer/BioNTech's cost of manufacturing these vaccines was roughly $14 per dose (106), and this is expected to decrease as the scale of production increases through 2021. This is consistent with our previous forecast that mRNA vaccines could be economically produced at scale, given the high-yield, enzymatic nature of mRNA synthesis and relatively simple purification process (3).

Notwithstanding the relative affordability of mRNA vaccines, access to COVID-19 mRNA vaccines has been greatly restricted by the pace of manufacturing and by barriers for many middle- and low-income countries to purchase vaccines. By April 2021, 4 months after the earliest vaccine authorizations, high-income countries had prepurchased 82% of vaccine doses from Pfizer/BioNTech and Moderna, with the United States and European Union accounting for 60% of the total (107). The COVID-19 Vaccines Global Access (COVAX) initiative was launched to direct vaccines to low-income countries, although the pace of vaccine procurement has been limited as of September 2021 (108). Besides additional donations through COVAX, one way that global access to mRNA vaccines could be boosted is through agreements to produce large quantities of vaccines in middle-income countries with high vaccine manufacturing capacity, such as India and Brazil, thereby increasing supply and reducing cost.

Booster Immunizations

As this review is in preparation, the provision of booster immunizations for people who have received the two-dose series of the Moderna and Pfizer/BioNTech vaccines is highly controversial.

Pfizer/BioNTech and Moderna have sought approval of a third immunization in non-immune-compromised individuals based on evidence of gradually waning vaccine efficacy against symptomatic COVID-19 and severe COVID-19 (in those >60 years old) during the rise of the Delta variant (82, 83, 88). Pfizer/BioNTech published impressive data showing a dramatic boost in neutralizing antibodies when 23 clinical trial participants were given a third dose of vaccine >6 months after dose 2: The ID_{50} titer against the Delta variant increased >5-fold in the 18–55-year-old group and >11-fold in the 65–85-year-old group between 1 month after dose 2 and 1 month after dose 3 (109). Even greater increases (>25-fold for wild type or Beta variant viruses) were observed when titers were compared between 8 months after dose 2 and 1 month after dose 3. Similar results were obtained by Moderna using a half-dose (50 μg) booster of its original mRNA-1273 vaccine (110). A retrospective study from Israel analyzed the health care records of 1.1 million individuals to determine the potential impact of a third dose of BNT162b2 in people aged 60 years or older (111). The authors found 11-fold fewer cases of confirmed SARS-CoV-2 infection and 20-fold fewer cases of severe COVID-19 in boosted people compared to nonboosted people between 12 days and no more than 4 weeks after dose 3. To account for potential confounding differences between the boosted and nonboosted populations, the authors also compared the rate of SARS-CoV-2 infection in the boosted group at time intervals before and after the vaccine efficacy is expected to be boosted by dose 3: They identified 5-fold fewer SARS-CoV-2 infections at ≥12 days after dose 3 compared to 4–6 days after dose 3. Given the scarce availability of COVID-19 vaccines in many countries (as of September 2021), the head of the World Health Organization has called for a moratorium on booster immunizations until at least the end of 2021, with the goal of securing vaccines to immunize the highest-risk segment of the global population that has not yet received their first dose (112). Despite this, many countries have recommended booster immunizations using mRNA-based or other vaccines, particularly in high-risk groups such as the elderly, immune-compromised, and those living in group homes.

Storage and Stability

A logistical challenge to the widespread deployment of COVID-19 mRNA vaccines is the temperature required for storage. While many vaccines are stable at 4°C for weeks or months, the Moderna vaccine requires −20°C for long-term storage and 4°C for up to 30 days, and the Pfizer/BioNTech vaccine originally required −80°C for long-term storage and 4°C for up to 5 days (prior to dilution with saline). Both vaccines must be used within several hours upon reaching room temperature. To our best knowledge, as of Sep. 30, 2021, no data have been made public to indicate whether these temperature requirements are strictly necessary to preserve the activity of the vaccines or if they were the only validated conditions at the time of submission to regulatory agencies. It is plausible that the stability differs due to the distinct lipid composition or to the different excipients added to the vials (113). Stability studies are by their nature very time-consuming to perform and difficult to optimize in a pandemic setting. However, in the first update to their storage requirements, Pfizer/BioNTech received approval to store its undiluted vaccines for up to 2 weeks at −20°C (114), thereby facilitating the logistics of transporting the vaccine and storing it prior to administration. Moderna is currently beginning phase I clinical studies of a new COVID-19 mRNA vaccine that is refrigerator stable (115), and Pfizer/BioNTech is testing a lyophilized formulation of BNT162b2 (NCT04816669).

FUTURE OUTLOOK

The future of mRNA vaccines is undoubtedly bright. The nucleoside-modified mRNA-LNP technology has proven to be an ideal platform for use in a pandemic setting due to its flexibility,

amenability to rapid mass production, strong record of immunogenicity, and now safety. Interestingly, CureVac's unmodified mRNA-LNP vaccine proved to be much less efficacious than the nucleoside-modified mRNA vaccines from Moderna and Pfizer/BioNTech (48.2% versus >90% in phase III studies). This raises the hypothesis that the unmodified mRNA-LNP platform could induce suboptimal neutralizing antibody responses in general; however, it is also possible that the lower vaccine dose (12 μg of mRNA for CureVac versus 30–100 μg for the others) or other factors such as circulating variants of concern contributed to the lower efficacy. Currently, no published clinical data are available on the safety and efficacy of self-amplifying COVID-19 mRNA vaccines. Another interesting question is whether the Moderna vaccine stimulates a longer-lived protective immune response than the Pfizer/BioNTech vaccine, as has been suggested (82), and whether this is due to the differing doses of mRNA (30 μg for Pfizer/BioNTech versus 100 μg for Moderna), the differing ionizable lipids, or other explanations. Future studies will answer these important questions.

Nucleoside-modified mRNA-LNP vaccines are already under development against a variety of infectious diseases beyond SARS-CoV-2 (11). However, fully realizing the promise of mRNA-based vaccines and therapies will require a great deal more research and development. Optimization of LNPs as carrier and adjuvant will require a better understanding of particle uptake, membrane fusion, and the mechanism(s) by which LNPs exert their adjuvant effect. It remains to be seen whether the pro-GC activity of ionizable LNPs can be dissociated from the inflammation that drives adverse events after vaccination. Another important area of research that will synergize with mRNA vaccines is structure-based, rational antigen design. COVID-19 mRNA vaccines took advantage of years of basic research that identified the S-2P mutations in MERS-CoV (10), and still further progress is being made in this area (116). Development of mRNA vaccines for more difficult viral targets, such as HIV, may require novel structure-guided approaches to achieve protective antibody responses. Likewise, advancing mRNA vaccines into the arena of cancer therapy may require innovations in our understanding of basic immunology and tumor biology.

Finally, despite the promise of mRNA vaccines, we caution that they are far from a silver bullet for future pandemics. Comprehensive pandemic preparedness (described in 117) requires significant new investments in viral surveillance, proactive clinical testing of vaccines for pandemic-potential viruses, new diagnostic technologies, broad-spectrum antiviral treatments (small-molecule inhibitors, immune modulators, etc.), and stockpiling of materials. Rapid and accurate diagnostics paired with broad-spectrum treatments could mitigate morbidity and mortality in a future pandemic while buying time for vaccine rollout, without the disruption of an economic shutdown. Antivirals could also be used for pre- or postexposure prophylaxis to prevent viral spread. Two forms this approach might take are a small-molecule inhibitor or an mRNA-encoded passive immunotherapy, for example, mRNA-LNPs encoding a cocktail of broadly neutralizing antibodies (118) against the viral family in question.

Looking forward, with the successful administration of over 1 billion doses of mRNA vaccines anticipated by late 2021, we expect that the barriers to entry for the development and clinical testing of future mRNA-based therapeutics will be much lower, not only for vaccines but also for a variety of other mRNA-based therapies.

DISCLOSURE STATEMENT

N.P. and M.J.H. are named on patents describing the use of modified mRNA in lipid nanoparticles as a vaccine platform. We have disclosed those interests fully to the University of Pennsylvania and Children's Hospital of Philadelphia, and we have in place an approved plan for managing any potential conflicts arising from licensing of our patents.

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