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mRNA Vaccine Design Modifications and Their Implications for Next-Generation Vaccines: Lessons from COVID-19

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Abstract

The COVID-19 pandemic accelerated rapid advances in vaccine development and underscored the need for platforms that can be rapidly engineered, highly effective, and adaptable to evolving pathogens. Messenger RNA (mRNA) vaccines have demonstrated exceptional efficacy and safety, establishing a significant milestone in modern vaccinology. This review aims to synthesize recent molecular engineering innovations that determine mRNA vaccine performance, focusing on nucleoside modification, spike protein prefusion stabilization, codon and untranslated region (UTR) optimization, and lipid nanoparticle (LNP) delivery systems. A literature search was conducted using PubMed and Google Scholar for studies published between 2020 and 2023, and selected publications were analyzed to identify key molecular determinants shaping vaccine function. Findings indicate that incorporation of N1-methyl-pseudouridine enhances mRNA stability, improves translational output, and reduces innate immune activation. The double-proline modification maintains the spike protein's prefusion conformation, while codon and UTR optimization further elevate antigen expression. LNP encapsulation provides structural protection and supports efficient intracellular delivery. Collectively, these engineering strategies enhance immunogenicity, ensure favorable tolerability, and allow rapid adaptation to emerging variants. Overall, integrated molecular design and advanced delivery technologies position mRNA vaccines as a transformative platform for next-generation vaccine development and strengthened global pandemic preparedness.

Keywords: COVID-19, mRNA vaccine, mRNA modification, LNP

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Introduction

The global health sector has faced extraordinary strain since the emergence of COVID-19 at the end of 2019. The disease, triggered by the newly recognized severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was initially detected in Wuhan, China, in December 2019 (Saddik et al., 2021). On 11 March 2020, the World Health Organization (WHO) officially recognized the rapid worldwide spread of COVID-19 and classified the outbreak as a global pandemic (World Health Organization, 2020). Clinically, individuals infected with the virus often experience manifestations resembling viral pneumonia, including elevated body temperature, general fatigue, and a persistent non-productive cough. In more serious circumstances, these initial symptoms can advance to complications such as acute respiratory distress, impairment of kidney function, or even failure of multiple organ systems (Cucinotta & Vanelli, 2020). Since the onset of the pandemic, various therapeutic strategies have been explored, including herbal preparations, antiviral agents such as remdesivir and favipiravir, and monoclonal antibody based therapies (Msemburi et al., 2023; World Health Organization, 2021). This discrepancy underscores the global impact of the pandemic and highlights the urgency of strengthening preventive measures, particularly widespread vaccination, to reduce transmission and mortality.

Since the beginning of the pandemic, numerous treatment approaches have been investigated, ranging from herbal-based remedies and antiviral drugs like remdesivir and favipiravir to therapies utilizing monoclonal antibodies. Nonetheless, their clinical effectiveness has remained limited. Randomized controlled trials have shown that favipiravir does not significantly improve

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recovery time, viral clearance, or disease progression compared with placebo in patients with mild to moderate COVID-19 (Udwadia et al., 2021). Consequently, vaccination has emerged as the most effective and sustainable approach for pandemic control. Large-scale clinical investigations have shown that mRNA vaccines markedly decrease the likelihood of progressing to severe disease or requiring hospitalization, with reported effectiveness surpassing 90% in adult cohorts (Adams et al., 2022; Ferdinands et al., 2022). Although immunity against mild infections diminishes with time, the vaccines continue to demonstrate strong and sustained protection against severe illness, underscoring their role as a fundamental component of global public health efforts (Saadh & Jaber, 2022).

Several vaccine platforms have been developed against COVID-19, including inactivated vaccines utilizing neutralized viral particles, protein subunit vaccines employing purified viral antigens, and viral vector vaccines using recombinant carrier viruses (Jiao, 2022). Among these, mRNA based vaccines have demonstrated exceptional performance owing to their rapid development, design flexibility, and high protective efficacy. These features position mRNA vaccines, exemplified by the Pfizer BioNTech and Moderna formulations, as a landmark advancement in modern vaccinology (Noor, 2021).

The remarkable success of mRNA vaccines stems from a series of structural and molecular engineering strategies designed to enhance their stability, efficiency, and safety. Key modifications include substitution of natural uridine (U) with ($m^1\Psi$) to suppress innate immune activation (Pardi et al., 2018), incorporation of double proline mutations (K986P and V987P) in the spike protein to preserve its prefusion conformation (Corbett et al., 2020), optimization of codons and UTR to improve transcript stability and translational efficiency (Corbett et al., 2020), and encapsulation within LNP that protect mRNA and facilitate cellular uptake (Polack et al., 2020). These molecular strategies collectively distinguish mRNA platforms from conventional vaccines, resulting in superior immunogenicity and adaptability (Starostina et al., 2021).

Building on these prior advances, this review provides an integrated evaluation of the structural engineering strategies incorporated into mRNA-based COVID-19 vaccines, including their effects on antigen expression, differences in design frameworks, and the refinement of LNP-facilitated delivery platforms. Moreover, the substantial scientific insights gained throughout the development of mRNA vaccines during the COVID-19 pandemic now serve as a strong foundation for creating next-generation vaccines directed at other infectious diseases, such as dengue, Zika, and influenza. With its inherent design flexibility, rapid production timeline, and adaptability to emerging variants, the mRNA platform serves as a strategic model for accelerating vaccine innovation and strengthening global preparedness for future outbreaks and pandemics.

Materials and Methods

The review was structured using the PICO framework, which consists of Population (P), Intervention (I), Comparator (C), and Outcome (O), to systematically define the research question and eligibility criteria. The population included preclinical models (in vitro and animal studies) and human participants enrolled in clinical trials. The interventions comprised molecular engineering strategies applied to mRNA vaccine design, including N1-methyl-pseudouridine ($m^1\Psi$) incorporation, 2P spike protein stabilization, codon optimization, UTR engineering, and LNP-based delivery systems. The comparators consisted of conventional vaccine platforms or unmodified mRNA constructs. The outcomes assessed in this review included the effects of these molecular modifications on mRNA stability, translational efficiency, antigen expression, immunogenicity, vaccine efficacy, and safety profiles, which were used to evaluate their contribution to the overall performance of mRNA vaccine platforms.

Eligibility was determined using predefined inclusion and exclusion criteria shown in Table 1. These criteria were used as

Table 1. Inclusion and Exclusion Criteria

Category	Criteria
Inclusion	– Studies examining molecular engineering of mRNA COVID-19 vaccines ($m^1\Psi$ substitution, 2P spike stabilization, codon optimization, UTR engineering, LNP delivery) – Preclinical or clinical studies providing mechanistic or immunological data. – Original research or clinical trial reports. – Published in English in international journals between 2020–2023.
Exclusion	– Not related to mRNA vaccine molecular engineering. – Lacking molecular or immunological relevance. – Commentary, opinion, or non–peer-reviewed articles. – Duplicate or overlapping data.

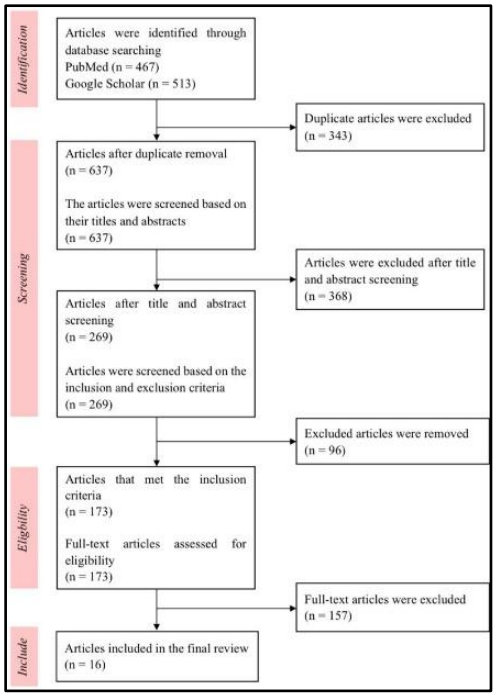

Figure 1. PRISMA Flow Diagram

the basis for selecting studies relevant to molecular engineering of mRNA COVID-19 vaccines.

A literature search was performed in PubMed and Google Scholar for publications from 2020 to 2023 using the terms *"COVID-19," "mRNA vaccine," "mRNA modification,"* and *"LNP."* All identified articles were organized using a reference management tool, with duplicate entries eliminated, after which titles and abstracts were reviewed and eligible studies were further evaluated through full-text analysis.

The study screening and selection adhered to PRISMA guidelines, as illustrated in the corresponding flow diagram (Figure 1). In total, 16 studies satisfied all predefined eligibility requirements and were subsequently incorporated into this review.

Data extraction was conducted manually, covering molecular design characteristics, delivery system components, mechanistic findings, and immunological outcomes. Due to variation in methods and reported results, the data were synthesized narratively.

Results and Discussion

Recent progress during the COVID-19 pandemic has positioned mRNA vaccines as one of the most transformative developments in modern vaccine science (Pardi et al., 2018; Park et al., 2021). These platforms deliver transient

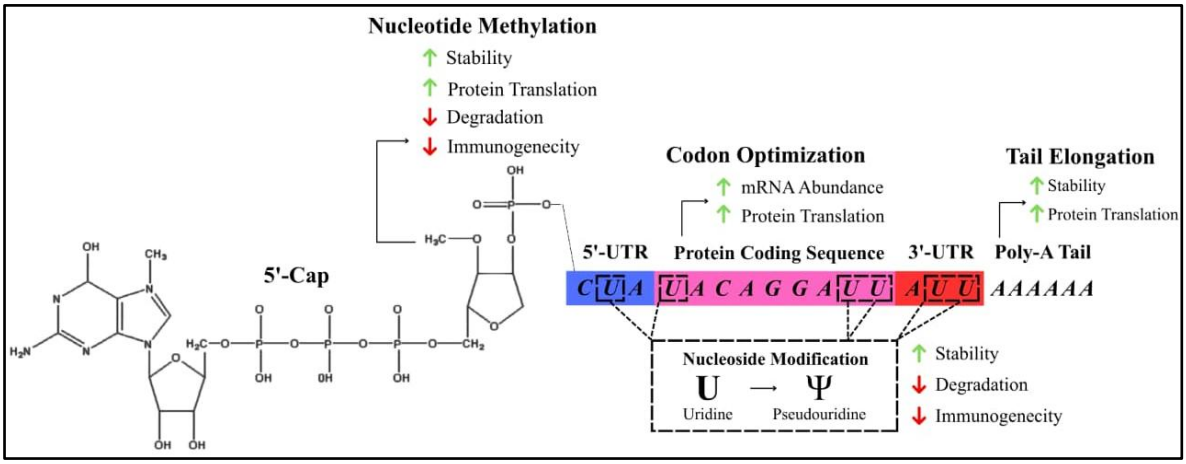

Figure 2. General structure of an mRNA vaccine construct and the key modification sites that enhance molecular stability, translational efficiency, and immunogenicity (adapted from Park et al., 2021).

genetic instructions into host cells, where they are translated into antigenic proteins, such as the SARS-CoV-2 spike protein, thereby inducing both humoral and cellular immune responses (Pardi et al., 2018; Sahin et al., 2020). Unlike conventional vaccine platforms that rely on inactivated pathogens or recombinant proteins, mRNA vaccines exploit host ribosomes to directly translate synthetic mRNA into antigenic proteins within the cytoplasm, eliminating the need for infectious viral material while closely mimicking natural antigen expression (Pardi et al., 2018; Park et al., 2021; Verbeke et al., 2021).

From a biological perspective, mRNA vaccine constructs are engineered to mimic endogenous eukaryotic transcripts, allowing them to be efficiently recognized by the host translational machinery. The mRNA construct consists of four essential structural elements: the 5′ cap, the 5′ and 3′ untranslated regions (UTRs), the open reading frame (ORF), and the poly(A) tail, each serving complementary roles in regulating transcript stability, translational efficiency, and antigen expression (Pardi et al., 2018; Verbeke et al., 2021). This molecular architecture enables synthetic mRNA to closely resemble endogenous transcripts while incorporating chemical modifications that enhance transcript stability, improve translational efficiency, and minimize excessive innate immune activation (Pardi et al., 2018; Starostina et al., 2021).

Therefore, the remarkable clinical performance of current mRNA vaccines results not simply from the encoded antigen sequence but from the coordinated integration of multiple molecular engineering strategies, each contributing to transcript stability, antigen expression, and immune activation (Pardi et al., 2018).

As illustrated in Figure 2, the mRNA vaccine construct incorporates multiple complementary structural modifications that collectively enhance transcript stability, translational efficiency, and immunogenicity. Accordingly, the contribution of each component should be interpreted as part of an integrated molecular engineering strategy rather than as an isolated structural modification.

As shown in Figure 2, each structural element of the mRNA construct performs a distinct yet complementary role, and their coordinated optimization collectively determines transcript stability, translational efficiency, and antigen expression. The 5′ cap protects the mRNA from enzymatic degradation while facilitating ribosome recognition and translation initiation. Further optimization of this region through the use of anti-reverse cap analogs (ARCA) or the CleanCap method has been shown to improve translational efficiency and prolong intracellular mRNA stability (Pardi et al., 2018; Starostina et al., 2021; Verbeke et al., 2021). Subsequently, the UTRs located at the 5′ and 3′ ends play a crucial role in regulating the stability and translational efficiency of mRNA. Optimization of these sequences through the selection of stable genetic elements, such as α-globin or β-globin motifs, has been shown to extend the cytoplasmic half-life of the transcript and enhance the production of antigenic proteins (Pardi et al., 2018; Verbeke et al.,

2021). The central ORF encodes the SARS-CoV-2 spike protein, which is engineered to maintain the prefusion conformation through incorporation of the 2P mutation. This stabilization preserves key neutralizing epitopes and enhances immune recognition (Corbett et al., 2020; Wrapp et al., 2020).

In addition, the poly(A) tail located at the 3′ end of the mRNA molecule plays a vital role in enhancing molecular stability and translational efficiency by preventing premature degradation. An optimal poly(A) tail length of approximately 100 to 150 nucleotides has been shown to strengthen the interaction with polyadenylate-binding proteins, thereby extending the functional lifespan of the mRNA transcript (Pardi et al., 2018). Beyond structural optimization of the poly(A) tail, transcript performance is further enhanced through nucleoside modification, in which uridine is replaced with $m^1\Psi$. This chemical modification reduces activation of innate immune receptors such as TLR7 and TLR8, thereby minimizing excessive immune activation while maintaining efficient antigen expression (Sahin et al., 2020). The entire system is subsequently encapsulated and delivered through LNP, which serve both as delivery vectors and as protective carriers that safeguard mRNA from enzymatic degradation by RNases. These nanoparticles also facilitate endosomal escape, allowing the mRNA to successfully reach the cytoplasm where efficient translation can occur (Sahin et al., 2020; Verbeke et al., 2021). These findings indicate that delivery efficiency depends not only on the presence of LNPs but also on the precise balance among lipid components, which collectively influences intracellular delivery, transcript stability, and overall vaccine performance (Verbeke et al., 2021).

These integrated molecular engineering strategies established the design framework adopted by first-generation mRNA vaccines, including BNT162b2 and mRNA-1273, both of which demonstrated high efficacy and favorable safety profiles in phase III clinical trials (Baden et al., 2021; Polack et al., 2020).

Importantly, the high clinical efficacy observed in first-generation mRNA vaccines cannot be attributed to a single structural modification but rather to the synergistic interaction among multiple molecular engineering strategies. While $m^1\Psi$ enhances transcript stability and reduces innate immune activation, optimal vaccine performance also depends on the 2P mutation and efficient intracellular delivery mediated by LNPs. Collectively, these coordinated modifications optimize transcript stability, preserve antigen conformation, and improve intracellular delivery, thereby maximizing antigen expression and the resulting adaptive immune response. Therefore, the clinical success of first-generation mRNA vaccines reflects the integrated outcome of coordinated molecular engineering rather than the contribution of any individual structural component alone (Corbett et al., 2020; Pardi et al., 2018).

Table 2. Comparative Data on Immunogenicity and Efficacy of mRNA COVID-19 Vaccines (Moderna mRNA-1273 and Pfizer BNT162b2)

Vaccine Type	mRNA Construct Modifications	Target Gene of Interest	Antigenicity Evaluation	Immunogenicity Evaluation		Vaccine Efficacy	Refer-ence
				Pre clinical	Clinical		
Moderna (mRNA-1273)	$m^1\Psi$; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	High neutralizing antibody response	BALB/c mice: Increased antibody & T-cell response	N=44.000 (≥18 years): Enhanced antibody & T-cell responses, mild adverse effects	Phase III: 94,1% (symptomatic)	(Noor, 2021)
Moderna (mRNA-1273)	$m^1\Psi$; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	Elevated specific IgG & neutralizing antibody titers	BALB/c mice: Increased antibody & T-cell responses	N=7.466, (≥65 years); N=22.885 (≥18 years): Enhanced antibody response, mild effects	Phase III: 94,1% (symptomatic); 100% (protection from severe cases)	(Depla nque & Launay , 2021)

Vaccine Type	mRNA Construct Modifications	Target Gene of Interest	Antigenicity Evaluation	Immunogenicity Evaluation		Vaccine Efficacy	Refer-ence
				Pre clinical	Clinical		
Moderna (mRNA-1273)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	Prefusion stabilized protein induces high neutralizing antibodies	BALB/c mice & *rhesus macaque*: Strong antibody response & complete protection without toxicity	N=30.415 (≥18 years): Mild moderate side effects, no myocarditis or pericarditis	Phase III: 93,2% (symptomatic) ; 98,2% (severe)	(El Sahly et al., 2022)
Moderna (mRNA-1273)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	Prefusion stabilized protein elicits strong neutralizing antibody response	BALB/c mice & *rhesus macaque*: High antibody levels, complete protection, no toxicity	N= 4.016 (6-11 years): Mild moderate effects, no myocarditis or MIS-C	Phase III: 88,0% (symptomatic)	(Creech et al., 2022)
Moderna (mRNA-1273)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	Prefusion stabilized protein induces potent neutralizing antibodies	BALB/c mice & *rhesus macaque*: Strong antibody response & full protection	N= 3.732 (12-17 years): Higher antibody titers than young adults, no myocarditis or MIS-C	Phase III: 93,3% (symptomatic)	(Ali et al., 2021)
Moderna (mRNA-1273)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	Not specified	*Rhesus macaque*: Increased antibody & T-cell responses, no lung lesions, no viral replication post-challenge	N= 30.420 (18-95 years): Strong antibody response, mild moderate effects	Phase III: 94,1% (symptomatic) ; 100% (protection from severe disease)	(Baden et al., 2021)
Moderna (mRNA-1273)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (SM-102, DSPC, Chol, PEG2000-DMG)	SARS-CoV-2 Spike (S) protein	High IgG & neutralizing antibody titers	*Rhesus macaque*: Elevated antibody levels & lung protection	N=60 (18-55 years): Strong antibody response, mild moderate effects	Phase I: High immunogenicity & safety demonstrated	(Jackson et al., 2020)
Pfizer (BNT162b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-	SARS-CoV-2 Spike (S) protein	Stable, highly expressed Spike protein	BALB/c mice & *rhesus macaque*: Full protection	N=43.548 (≥16 years): Increased antibody response, mild	Phase III: 95% (symptomatic)	(Vogel et al., 2021)

Vaccine Type	mRNA Construct Modifications	Target Gene of Interest	Antigenicity Evaluation	Immunogenicity Evaluation		Vaccine Efficacy	Refer-ence
				Pre clinical	Clinical		
	0159, DSPC, Cholesterol)			without pathology	moderate side effects		
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Induces neutralizing antibodies & $CD4^+/CD8^+$ T-cell responses	Not specified	N= 43.448 (≥16 years): Mild moderate effects	Phase III: 95% (symptomatic)	(Polack et al., 2020)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Elevated IgG & neutralizing antibody titers	BALB/c mice: Enhanced neutralization activity	N= 15.912 (>55 years); N=21.794 (≥16 years): Strong antibody & neutralization responses, mild effects	Phase III: 95% (symptomatic); 100% (severe protection)	(Deplanque & Launay, 2021)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	High prefusion-stabilized Spike expression & neutralizing antibody production	BALB/c mice & *rhesus macaque*: Full protection, no immunopathology	N=44.165 (≥16 years); N=2.264 (12-15 years): Mild moderate effects, no severe adverse events	Phase III: 91.3% (severe cases)	(Thomas et al., 2021)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Prefusion-stabilized Spike protein induces potent neutralizing antibodies	BALB/c mice & *rhesus macaque*: Strong antibody response, full protection, no toxicity	N=2.260 (12-15 years): Antibody titers higher than adults, mild moderate effects	Phase III: 95% (symptomatic)	(Frenck et al., 2021)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Stable prefusion Spike induces strong neutralizing antibodies	BALB/c mice & *rhesus macaque*: High antibody titers and safety confirmed	N=2.268 (5-11 years): Mild moderate effects, antibody levels comparable to adults	Phase III: 90,7% (symptomatic)	(Rodríguez-Chávez et al., 2023)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Prefusion-stabilized, highly expressed Spike induces strong neutralizing antibodies	BALB/c mice & *rhesus macaque*: High antibody titers, full protection without toxicity	N=10.125 (≥16 years): Mild moderate effects, no myocarditis or pericarditis	Phase III: 95,3% (symptomatic)	(Moreira et al., 2022)

Vaccine Type	mRNA Construct Modifications	Target Gene of Interest	Antigenicity Evaluation	Immunogenicity Evaluation		Vaccine Efficacy	Refer-ence
				Pre clinical	Clinical		
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	High neutralizing antibody response	BALB/c mice: Increased antibody response	(16 years): Elevated antibodies, mild effects	Phase III: >90% (symptomatic)	(Noor, 2021)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Not specified	Not specified	N= 596.618 (≥16 years): Mild effects	Phase III: 94% (symptomatic) ; >90% (severe, hospitalized cases)	(Dagan et al., 2021)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Enhanced neutralizing antibody titers	Not specified	N=60 (18-55 years): Levated RBD & neutralizing antibodies, increased $CD4^+$ & $CD8^+$ T-cell responses, mild moderate side effects	Phase I/II: High immunogenicity, strong protective potential	(Sahin et al., 2020)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Increased RBD & neutralizing antibodies	Not specified	N=45 (18-55 years): High antibody titers, mild moderate effects	Phase I/II: High immunogenicity & safety	(Mulligan et al., 2020)
Pfizer (BNT162 b1)	m¹Ψ; S-2P; UTR; Poly (A) Tail; 5' Cap; LNP (ALC-0315, ALC-0159, DSPC, Cholesterol)	SARS-CoV-2 Spike (S) protein	Increased RBD & neutralizing antibodies	Not specified	N=45 (18-55 years): High antibody titers, mild moderate effects	Phase I/II: High immunogenicity & safety	(Mulligan et al., 2020)

Abbreviations: RBD, receptor-binding domain; LNP, lipid nanoparticle; UTR, untranslated region; S-2P, prefusion-stabilized spike protein containing two proline substitutions; m¹Ψ, N1-methylpseudouridine; IgG, immunoglobulin G; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; PEG, polyethylene glycol; MIS-C, multisystem inflammatory syndrome in children.

In contemporary immunology and biotechnology, mRNA vaccine platforms have emerged as a significant innovation because they use synthetic genetic instructions to direct host cells to synthesize target antigens without relying on live or infectious viral material. In this approach, an engineered mRNA sequence encoding the SARS-CoV-2 spike protein is delivered into host cells, where it is translated by host ribosomes into antigenic spike proteins. These intracellular antigens are then recognized by the immune system as foreign, prompting a protective response characterized by the production of neutralizing antibodies and the activation of $CD4^+$ helper T cells together with $CD8^+$ cytotoxic T cells (Corbett et al., 2020; Pardi et al., 2018; Sahin et al., 2020).

From a structural perspective, mRNA vaccines are engineered to mimic endogenous eukaryotic transcripts through coordinated

structural elements, including the 5′ cap, 5′ and 3′ UTRs, the open reading frame (ORF), and the poly(A) tail. Together, these components maintain transcript stability, promote efficient translation, and maximize antigen expression, thereby enabling robust activation of adaptive immune responses (Pardi et al., 2018; Verbeke et al., 2021).

Various COVID-19 vaccine platforms, including viral vector, protein subunit, inactivated virus, and DNA vaccines, have demonstrated protective efficacy through different mechanisms of antigen delivery and immune activation. Reported efficacy against symptomatic COVID-19 generally ranges from approximately 65%–90%, while protection against severe disease remains consistently high across platforms (Dai et al., 2022; Heath et al., 2021; Khobragade et al., 2022; Logunov et al., 2021; Sadoff et al., 2021; Tanriover et al., 2021; Voysey et al., 2021). These observations indicate that differences in vaccine efficacy are influenced not only by the antigen itself but also by the underlying delivery platform and molecular design strategy.

Compared to other vaccine technologies, mRNA vaccines demonstrate superior immunological potency and biological efficiency. This platform functions directly within the cytoplasm without requiring transcription in the cell nucleus, thereby enabling rapid and robust antigen production (Pardi et al., 2018; Sahin et al., 2020). Clinical trials have shown that mRNA vaccines achieve efficacy rates of approximately 94–95% against symptomatic infection and provide nearly complete protection against severe disease, consistent with the data summarized in Table 2 (Baden et al., 2021; Polack et al., 2020). In addition to their high clinical efficacy, mRNA vaccines elicit markedly stronger neutralizing antibody titers than other vaccine platforms and induce potent $CD4^+$ and $CD8^+$ T-cell activation. The immune response is predominantly Th1-oriented, offering protection against antibody-dependent enhancement (ADE) and ensuring a balanced and durable adaptive immunity (Corbett et al., 2020; Sahin et al., 2020).

However, the superior efficacy of mRNA vaccines should not be interpreted solely as a consequence of the mRNA platform itself. Instead, it reflects the integration of optimized antigen design, molecular engineering, and advanced delivery technology. As summarized in Table 2, both BNT162b2 and mRNA-1273 employ the same core design principles, including $m^1\Psi$ substitution, 2P spike stabilization, and lipid nanoparticle formulation, despite differences in lipid composition. The comparable efficacy achieved by both vaccines suggests that coordinated molecular engineering, rather than any individual structural modification, is the primary determinant of vaccine performance (Baden et al., 2021; Pardi et al., 2018). Although viral vector and protein-based vaccines can also induce protective immunity, their performance may be influenced by factors such as pre-existing vector immunity, slower antigen redesign, or more complex manufacturing processes. Therefore, the principal advantage of mRNA technology lies not only in its immunogenic potency but also in its flexibility, rapid adaptability, and scalability during emerging infectious disease outbreaks.

In addition to high efficacy, mRNA vaccines exhibit favorable safety profiles because they are non-replicating, contain no infectious material, and do not integrate into the host genome. Their rapid manufacturing process and adaptability to emerging variants further distinguish this platform from conventional vaccine technologies (Pardi et al., 2018; Sahin et al., 2020).

Despite these advantages, native mRNA remains inherently unstable and is highly susceptible to RNase-mediated degradation. Consequently, multiple molecular engineering strategies, including chemical modification, sequence optimization, and advanced delivery systems, are required to improve transcript stability, translational efficiency, and overall vaccine performance (Pardi et al., 2018; Verbeke et al., 2021).

Collectively, the molecular modifications summarized in Table 2 demonstrate that the exceptional performance of current mRNA vaccines results from the integration of complementary engineering strategies rather than any single design element. These findings provide the basis for the following discussion of how individual structural components contribute to the molecular optimization and clinical performance of mRNA vaccines (Pardi et al., 2018; Verbeke et al., 2021).

Nucleoside Modification ($m^1\Psi$)

One of the most fundamental innovations driving the success of mRNA-based vaccines is the introduction of nucleoside modification, achieved by substituting natural uridine (U) with $m^1\Psi$ in the synthetic mRNA strand. This strategy was first shown to markedly reduce excessive innate immune activation triggered when in vitro-transcribed (IVT) mRNA is recognized by host pattern-recognition receptors (PRRs) (Pardi et al., 2018). Unmodified IVT mRNA contains uridine (U) residues that are readily recognized by innate immune sensors, including Toll-like receptors (TLR3, TLR7, and TLR8) and cytoplasmic RNA sensors such as RIG-I and MDA5. This recognition rapidly initiates type I interferon signaling and subsequent inflammatory cascades, leading to accelerated mRNA degradation and inhibition of antigen protein translation (Pardi et al., 2018).

By replacing natural uridine (U) with $m^1\Psi$, the mRNA molecule becomes less detectable by innate immune sensors without compromising ribosomal recognition and translation. Structurally, $m^1\Psi$ enhances base-pair stability and optimizes mRNA secondary structure, thereby improving transcript stability and translational efficiency. Reduced activation of TLR7 and TLR8 subsequently decreases type I interferon (IFN-α/β) production and limits pro-inflammatory cytokine release (Pardi et al., 2018; Sahin et al., 2020).

Beyond attenuating innate immune sensing, $m^1\Psi$ also reduces susceptibility to RNase-mediated degradation, thereby prolonging intracellular persistence and sustaining antigen expression. Prolonged antigen expression subsequently promotes stronger adaptive immune responses characterized by high neutralizing antibody titers and balanced $CD4^+$ and $CD8^+$ T-cell activation (Polack et al., 2020; Sahin et al., 2020). Clinical trials of first-generation mRNA vaccines incorporating $m^1\Psi$ modification demonstrated remarkable efficacy, achieving approximately 94–95% protection against symptomatic infection and nearly complete protection against severe cases, while maintaining a favorable safety profile with minimal reactogenicity (Baden et al., 2021; Polack et al., 2020).

Collectively, these findings demonstrate that $m^1\Psi$ modification simultaneously improves mRNA stability, translational efficiency, and immune compatibility, thereby establishing an optimal balance between antigen expression and controlled innate immune activation. These observations indicate that successful mRNA vaccine design depends not only on maximizing antigen production but also on precisely regulating host innate immune recognition. Consequently, $m^1\Psi$ has become a fundamental molecular feature of clinically successful mRNA vaccine platforms (Pardi et al., 2018; Verbeke et al., 2021).

Although $m^1\Psi$ modification substantially improves transcript stability and translation, optimal vaccine performance also requires structural optimization of the encoded antigen. Accordingly, subsequent advances focused on engineering the spike protein to preserve its prefusion conformation, thereby further enhancing immunogenicity (Corbett et al., 2020; Wrapp et al., 2020).

2P Spike Protein Stabilization Mutation (K986P and V987P)

The double proline substitution at residues K986 and V987, commonly referred to as the 2P mutation, represents one of the key structural engineering strategies underlying the success of current mRNA vaccines. This modification aims to preserve the spike glycoprotein in its prefusion conformation, the structural state preceding membrane fusion that exhibits the highest immunogenicity and relevance for neutralizing antibody recognition (Wrapp et al., 2020). This strategy was first reported by Wrapp et al. (2020), who demonstrated through cryo-electron microscopy (cryo-EM) that substitution of residues K986 and V987 with proline stabilizes the spike protein by maintaining its prefusion conformation.

At the molecular level, the rigid nature of proline residues restricts peptide bond rotation within the fusion subunit (S2 region) of the spike protein, thereby enhancing the thermodynamic stability of the molecule and preserving the native prefusion epitopes preferentially recognized by neutralizing antibodies. This conformational stability ensures that the antigen translated from mRNA within host cells closely resembles the native prefusion structure of the viral spike protein, resulting in more efficient and targeted

activation of adaptive immune responses (Corbett et al., 2020; Wrapp et al., 2020).

Preclinical studies consistently demonstrated that incorporation of the 2P mutation resulted in markedly higher neutralizing antibody titers and enhanced $CD4^+$ and $CD8^+$ T-cell responses compared with non-stabilized spike constructs, indicating that preservation of the prefusion conformation is essential for optimal antigen presentation (Corbett et al., 2020; Sahin et al., 2020). Moreover, this structural design directs the immune response toward a Th1-dominant profile, characterized by secretion of interferon-γ (IFN-γ) and interleukin-2 (IL-2), thereby reducing the risk of antibody-dependent enhancement (ADE) associated with excessive Th2-dominated responses (Sahin et al., 2020). The enhanced immunogenicity observed in preclinical studies was subsequently validated in Phase III clinical trials, where mRNA vaccines incorporating the 2P-stabilized spike antigen induced high-affinity neutralizing antibodies and demonstrated clinical efficacy exceeding 90% against symptomatic COVID-19 while maintaining strong protection against severe disease (Baden et al., 2021; Polack et al., 2020).

Although the 2P mutation effectively preserves the prefusion conformation, mutations arising within the receptor-binding domain (RBD) of emerging SARS-CoV-2 variants can reduce antibody recognition despite maintenance of the stabilized structure. These findings highlight that structural stabilization alone is insufficient to maintain vaccine performance against continuously evolving viral variants, thereby motivating the development of next-generation constructs such as HexaPro, which incorporates four additional proline substitutions to further enhance structural stability without compromising antigenicity (Wrapp et al., 2020).

Collectively, these findings demonstrate that the 2P mutation represents a cornerstone of mRNA vaccine antigen engineering by preserving the prefusion spike conformation required for optimal immune recognition. However, its contribution to vaccine performance is achieved through synergistic interactions with other molecular engineering strategies rather than as an isolated structural modification, providing the foundation for the development of next-generation mRNA vaccine antigens (Baden et al., 2021; Corbett et al., 2020; Polack et al., 2020; Sahin et al., 2020; Wrapp et al., 2020). Although structural stabilization through the 2P mutation substantially enhances antigenicity, optimal vaccine performance also depends on efficient mRNA translation. Accordingly, subsequent engineering strategies focused on codon optimization and UTR design to further improve transcript stability and antigen expression within host cells (Pardi et al., 2018).

Codon Optimization and UTR

In addition to nucleoside modification and spike protein engineering, the performance of mRNA vaccines is further optimized through codon optimization and UTR engineering, both of which maximize translational efficiency and transcript stability. Codon optimization involves modifying the mRNA coding sequence to match the codon usage preferences of human cells, which are influenced by the relative abundance of host transfer RNAs (tRNAs). The primary objective is to replace rarely used codons with synonymous codons that are more efficiently recognized by the host translational machinery, thereby reducing ribosomal pausing and improving translational efficiency (Pardi et al., 2018; Verbeke et al., 2021).

In addition to increasing translation speed, codon optimization also influences mRNA secondary structure, thereby improving ribosome accessibility and translation initiation while preserving efficient elongation. An optimal codon arrangement maintains a balance between mRNA structural stability and accessibility of the translation initiation region. Studies by Pardi et al. (2018) and Sahin et al. (2020) indicate that codon patterns aligned with human tRNA abundance markedly improve antigen expression and generate stronger humoral responses compared with constructs retaining native viral codons. These findings indicate that codon optimization improves vaccine performance not only by accelerating protein synthesis but also by maximizing antigen availability for immune recognition.

Furthermore, the UTRs located at the 5′ and 3′ ends of the mRNA play essential regulatory roles in controlling transcript stability and translational efficiency. UTRs influence translational factor binding, mRNA folding, and degradation rates within the

cytoplasm. Engineering the 5′ and 3′ UTRs through the incorporation of stable regulatory sequences derived from α-globin or β-globin genes has been reported to extend mRNA half-life and improve translational performance (Pardi et al., 2018; Verbeke et al., 2021). Optimized 5′ UTRs enhance ribosome recruitment and translation initiation, whereas stable 3′ UTRs reduce degradation and strengthen interactions with polyadenylate-binding proteins, thereby maintaining transcript integrity (Pardi et al., 2018; Verbeke et al., 2021).

Collectively, codon optimization and UTR engineering act synergistically to maximize antigen expression by simultaneously increasing translational efficiency and prolonging mRNA stability. These observations demonstrate that efficient antigen production depends not only on the encoded protein sequence but also on optimization of the regulatory elements controlling mRNA expression. Consequently, potent immune responses can be achieved with lower mRNA doses, thereby improving vaccine immunogenicity while maintaining formulation efficiency (Pardi et al., 2018; Sahin et al., 2020). Within modern mRNA vaccine platforms, codon optimization and UTR engineering complement nucleoside modification and spike protein stabilization to establish a rapid, robust, and durable antigen expression system (Pardi et al., 2018; Verbeke et al., 2021).

Although codon optimization and UTR engineering substantially enhance intracellular antigen expression, optimal translation also depends on protecting the mRNA transcript from degradation. Consequently, further optimization focuses on the 5′ cap and poly(A) tail, which stabilize the transcript and facilitate efficient translation initiation (Pardi et al., 2018; Verbeke et al., 2021).

Poly(A) Tail and 5′ Cap

The two terminal structural elements of mRNA, namely the 5′ cap and the poly(A) tail, play indispensable roles in preserving transcript stability and maximizing translational efficiency (Pardi et al., 2018; Verbeke et al., 2021). The poly(A) tail protects the 3′ end of the mRNA from degradation by exonucleases and extends its half-life within the cytoplasm. In addition to protecting the transcript from degradation, the poly(A) tail enhances translational efficiency through interactions with poly(A)-binding protein (PABP), which bridges the 3′ poly(A) tail with the translation initiation complex via eIF4G. This interaction promotes formation of a closed-loop mRNA structure that facilitates ribosome recycling and repeated initiation of translation, thereby increasing the total protein yield from a single mRNA molecule (Pardi et al., 2018; Park et al., 2021; Verbeke et al., 2021).

The length of the poly(A) tail is a key determinant of both stability and translational capacity. Experimental evidence indicates that a poly(A) tail of approximately 100–150 nucleotides provides an optimal balance between transcript stability and translational efficiency. Shorter tails undergo rapid deadenylation, whereas excessively long tails may interfere with mRNA folding and ribosome progression (Pardi et al., 2018; Sahin et al., 2020). The enzymatic properties of the poly(A) polymerase used during in vitro transcription also influence the uniformity of tail length, thereby contributing to consistent antigen expression across cells (Park et al., 2021; Verbeke et al., 2021).

At the 5′ end, the cap structure plays a crucial role in shielding the mRNA from exonuclease-mediated degradation and functions as a key molecular marker required for ribosome recognition during translation initiation. Native eukaryotic mRNA contains either cap0 or cap1 structures consisting of a 7-methylguanosine linked to the first nucleotide through a 5′–5′ triphosphate bridge. In current mRNA vaccine designs, additional structural refinements are incorporated to ensure correct cap orientation and strengthen interactions with the initiation factor eIF4E. Among these improvements, the anti-reverse cap analog (ARCA) and CleanCap methodologies are widely implemented because they preserve correct 5′ orientation and minimize generation of nonfunctional reverse caps (Pardi et al., 2018; Sahin et al., 2020; Starostina et al., 2021; Verbeke et al., 2021).

Implementation of ARCA and CleanCap substantially improves translational efficiency and extends mRNA half-life by ensuring correct cap orientation and enhancing recognition by the translation initiation

machinery (Pardi et al., 2018; Verbeke et al., 2021). Furthermore, advances in co-transcriptional capping technologies during in vitro transcription have enabled synthesis of high-purity, correctly oriented capped mRNA, reducing the need for extensive post-transcriptional purification steps (Park et al., 2021; Verbeke et al., 2021). These observations indicate that successful mRNA vaccine design depends not only on maximizing antigen production but also on preserving transcript integrity throughout intracellular translation.

Collectively, optimization of the poly(A) tail and the 5′ cap illustrates that efficient antigen expression depends not only on the coding sequence but also on precise regulation of transcript stability and translation initiation. Together with nucleoside modification, spike protein stabilization, and codon optimization, these terminal structural elements form an integrated molecular engineering strategy underlying the high performance of current mRNA vaccines. Following optimization of the mRNA construct itself, the next critical step involves encapsulation within LNPs, which protect the transcript from extracellular degradation and enable efficient intracellular delivery (Pardi et al., 2018; Park et al., 2021; Verbeke et al., 2021).

Delivery System: LNP

Lipid nanoparticles (LNPs) constitute the final and indispensable component of contemporary mRNA vaccine design, protecting the inherently unstable mRNA from extracellular RNase-mediated degradation while enabling efficient intracellular delivery to the cytoplasm, where antigen translation occurs. LNPs enclose the mRNA within lipid-based particles that remain neutral under physiological conditions but acquire a positive charge in the acidic environment of endosomes. This shift in charge promotes fusion with the endosomal membrane and enables the mRNA to enter the cytoplasm through the process of endosomal escape (Sahin et al., 2020; Verbeke et al., 2021).

Ionizable lipids are the most important component because they electrostatically complex with negatively charged mRNA during nanoparticle formation while remaining electrically neutral at physiological pH, thereby minimizing systemic toxicity. Following endocytosis, acidification of the endosome protonates these lipids, destabilizing the endosomal membrane and facilitating cytoplasmic release of mRNA. Lipids such as SM-102 and ALC-0315 exemplify this design strategy by balancing delivery efficiency, structural stability, and safety (Park et al., 2021; Verbeke et al., 2021).

Cholesterol contributes to structural stability by reinforcing the integrity of the lipid bilayer and enhancing membrane fluidity, which is essential for fusion with cellular membranes. DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) maintains the physical stability and rigidity of the nanoparticle bilayer, ensuring its robustness during storage and circulation. PEG-lipids, consisting of polyethylene glycol chains conjugated to lipid anchors, improve colloidal stability and prevent particle aggregation. Collectively, these structural lipids complement ionizable lipids by maintaining nanoparticle integrity throughout formulation, circulation, and cellular uptake, thereby maximizing the efficiency of intracellular mRNA delivery (Park et al., 2021; Verbeke et al., 2021).

Beyond lipid composition, nanoparticle size also represents an important determinant of LNP performance. An optimal particle size must balance systemic distribution with efficient uptake by antigen-presenting cells, with nanoparticles measuring approximately 80–100 nm generally exhibiting favorable characteristics for cellular internalization (Verbeke et al., 2021).

Beyond their protective role, LNPs also exhibit intrinsic immunological activity that supports vaccine efficacy. Certain ionizable lipids have been reported to act as adjuvants, stimulating dendritic cells and enhancing antigen presentation via both MHC class I and class II pathways. This activation leads to robust $CD4^+$ and $CD8^+$ T-cell responses, contributing to the establishment of long-lasting protective immunity (Sahin et al., 2020; Verbeke et al., 2021). These findings indicate that LNPs function not only as delivery vehicles but also as active immunomodulatory components that contribute directly to vaccine efficacy.

Although Moderna mRNA-1273 and Pfizer BNT162b2 differ in their proprietary lipid compositions, both formulations employ similar fundamental LNP design principles. Their comparable clinical efficacy suggests that

overall nanoparticle architecture and intracellular delivery efficiency are more important determinants of vaccine performance than the identity of individual lipid components alone (Pardi et al., 2018; Park et al., 2021; Verbeke et al., 2021). Collectively, these observations demonstrate that LNP formulations serve not merely as passive carriers but as active determinants of mRNA vaccine efficacy, safety, and immunogenicity.

Despite these advances, LNP technology remains one of the principal challenges limiting the broader application of mRNA therapeutics. Current formulations still require improvements in biodistribution, thermostability, reactogenicity, and tissue-specific targeting. Addressing these limitations will facilitate the expansion of mRNA technology beyond COVID-19 vaccines toward next-generation vaccines and a wide range of therapeutic applications, including cancer immunotherapy and protein replacement therapy (Park et al., 2021; Verbeke et al., 2021).

All molecular engineering strategies discussed previously, including nucleoside modification, spike protein stabilization, codon and UTR optimization, and LNP-based delivery, act as interconnected components that collectively contribute to mRNA vaccine performance. Rather than acting independently, these modifications form an integrated molecular framework that improves transcript stability, enhances antigen expression, regulates innate immune activation, and facilitates efficient intracellular delivery, ultimately contributing to strong and balanced immune responses (Pardi et al., 2018; Verbeke et al., 2021).

The clinical relevance of this integrated design is demonstrated by the immunogenicity, efficacy, and safety outcomes summarized in Table 2. Both first-generation mRNA vaccines, mRNA-1273 and BNT162b2, incorporated similar fundamental engineering principles, including $m^1\Psi$-modified nucleosides, prefusion-stabilized spike antigens through the 2P mutation, and LNP-based delivery systems. Despite differences in formulation characteristics, both vaccines consistently induced high neutralizing antibody responses, strong T-cell activation, and clinical efficacy exceeding 90% against symptomatic COVID-19 infection in phase III clinical trials (Baden et al., 2021; Polack et al., 2020).

Beyond their immunogenic performance, both vaccines demonstrated favorable safety profiles, with most reported adverse events being mild to moderate and transient, including injection-site reactions, fatigue, fever, and myalgia. Importantly, no major safety concerns that limited clinical implementation were identified during the evaluated clinical periods. These findings indicate that molecular optimization strategies successfully achieved a balance between efficient antigen expression and controlled immune activation (Baden et al., 2021; Polack et al., 2020).

The comparable performance observed between mRNA-1273 and BNT162b2 highlights that vaccine success cannot be attributed to a single structural modification but rather on the coordinated interaction of multiple engineering strategies. Modified nucleosides contribute to transcript stability and reduced innate immune sensing, spike stabilization preserves antigenic epitopes required for effective immune recognition, while LNP formulations ensure protection and intracellular delivery of mRNA. Therefore, the exceptional performance of current mRNA vaccines represents the cumulative outcome of optimized RNA chemistry, antigen design, and delivery technology rather than the contribution of an individual molecular component alone (Corbett et al., 2020; Pardi et al., 2018; Sahin et al., 2020).

The success of mRNA vaccines during the COVID-19 pandemic represents a transition from conventional empirical vaccine development toward rational molecular engineering. The ability to rapidly redesign genetic sequences and optimize vaccine components provides a flexible platform with potential applications against other infectious diseases, including influenza, Zika virus, dengue, and HIV, as well as emerging nucleic acid-based therapeutic approaches (Pardi et al., 2018; Verbeke et al., 2021).

Future development of mRNA vaccine technology is directed toward improving dose efficiency, stability, and global accessibility. One promising approach is the development of self-amplifying mRNA (saRNA), which incorporates replication elements derived from RNA viruses to enhance intracellular antigen production while potentially reducing the required vaccine dose. This strategy may

improve manufacturing efficiency and enhance the scalability of mRNA-based platforms (Sahin et al., 2020). In parallel, thermostable mRNA formulations are being investigated to overcome cold-chain limitations and facilitate broader vaccine distribution, particularly in resource-limited regions (Park et al., 2021; Verbeke et al., 2021).

Another emerging direction in mRNA vaccine development involves multivalent and combinatorial vaccine designs capable of encoding multiple antigenic targets within a single formulation. This strategy is particularly relevant for pathogens with high antigenic diversity, such as dengue virus, which consists of four distinct serotypes (DENV-1–DENV-4). Advances in computational biology and structural vaccinology enable rational antigen selection and optimization to improve cross-serotype immune recognition and enhance the breadth and durability of protective responses. However, successful development of multivalent mRNA vaccines requires careful optimization of antigen expression levels, potential immune interference, and formulation complexity.

Despite the remarkable progress of mRNA vaccine technology, several challenges remain across the five major molecular engineering strategies. Nucleoside modification requires further optimization to achieve an appropriate balance between minimizing excessive innate immune activation and maintaining sufficient immunogenic stimulation. Structural antigen engineering must be continuously refined to address viral evolution and mutations that may affect antigenic epitopes and immune recognition. Similarly, codon optimization and UTR engineering require precise sequence design to enhance translational efficiency while preserving proper mRNA folding and structural stability. Optimization of the 5′ cap and poly(A) tail remains critical for maintaining transcript integrity, prolonging mRNA stability, and ensuring efficient translation initiation. In addition, LNP formulations require further improvement in biodistribution, storage stability, cold-chain independence, and reduction of reactogenicity. Addressing these challenges requires integrated advances in RNA chemistry, computational antigen design, lipid engineering, and formulation technologies. Specifically, improved nucleoside chemistry may enhance the balance between innate immune regulation and antigen expression, while advanced computational approaches can facilitate accurate antigen prediction and optimization. Furthermore, next-generation delivery systems may improve LNP stability, targeted tissue delivery, and global vaccine accessibility.

Collectively, the advancement of mRNA vaccine technology demonstrates that successful vaccine performance depends on the coordinated optimization of RNA structure, antigen design, and delivery systems rather than antigen selection alone. By integrating molecular engineering strategies with computational and formulation approaches, next-generation mRNA vaccines have the potential to achieve improved efficacy, enhanced stability, broader pathogen coverage, and expanded biomedical applications beyond COVID-19.

Conclusion

Learning from the COVID 19 pandemic, the remarkable success of mRNA based vaccines has ushered in a new paradigm in modern vaccinology. This achievement demonstrates that precise molecular engineering, including nucleoside modification, structural stabilization of antigens, optimization of genetic sequences, and advanced delivery systems, can produce vaccines that are highly effective, safe, and rapidly developed. The scientific principles established during the development of COVID 19 vaccines have not only proven their efficacy against SARS CoV 2 but have also provided a conceptual foundation for creating vaccines against other infectious diseases such as influenza, Zika, and HIV, as well as potential applications in cancer immunotherapy.

Looking ahead, the design strategies and technological insights gained from the COVID 19 experience will guide the development of next generation vaccines that are more stable, adaptable, and globally scalable. Thus, the COVID 19 pandemic serves as a pivotal turning point that marks the beginning of a new era in mRNA based vaccinology, with the potential to revolutionize future strategies for disease prevention worldwide.

References

Adams, K., Rhoads, J. P., Surie, D., Gaglani, M., Ginde, A. A., McNeal, T., Talbot, H. K., Casey, J. D., Zepeski, A., Shapiro, N. I., Gibbs, K. W., Files, D. C., Hager, D. N., Frosch, A. E., Exline, M. C., Mohamed, A., Johnson, N. J., Steingrub, J. S., Peltan, I. D., … Self, W. H. (2022). Vaccine effectiveness of primary series and booster doses against covid-19 associated hospital admissions in the United States: Living test negative design study. *The BMJ*. https://doi.org/10.1136/bmj-2022-072065

Ali, K., Berman, G., Zhou, H., Deng, W., Faughnan, V., Coronado-Voges, M., Ding, B., Dooley, J., Girard, B., Hillebrand, W., Pajon, R., Miller, J. M., Leav, B., & McPhee, R. (2021). Evaluation of mRNA-1273 SARS-CoV-2 Vaccine in Adolescents. *New England Journal of Medicine*, *385*(24), 2241–2251. https://doi.org/10.1056/nejmoa2109522

Baden, L. R., El Sahly, H. M., Essink, B., Kotloff, K., Frey, S., Novak, R., Diemert, D., Spector, S. A., Rouphael, N., Creech, C. B., McGettigan, J., Khetan, S., Segall, N., Solis, J., Brosz, A., Fierro, C., Schwartz, H., Neuzil, K., Corey, L., … Zaks, T. (2021). Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *New England Journal of Medicine*, *384*(5), 403–416. https://doi.org/10.1056/nejmoa2035389

Corbett, K. S., Edwards, D. K., Leist, S. R., Abiona, O. M., Boyoglu-Barnum, S., Gillespie, R. A., Himansu, S., Schäfer, A., Ziwawo, C. T., DiPiazza, A. T., Dinnon, K. H., Elbashir, S. M., Shaw, C. A., Woods, A., Fritch, E. J., Martinez, D. R., Bock, K. W., Minai, M., Nagata, B. M., … Graham, B. S. (2020). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature*, *586*(7830), 567–571. https://doi.org/10.1038/s41586-020-2622-0

Corbett, K. S., Flynn, B., Foulds, K. E., Francica, J. R., Boyoglu-Barnum, S., Werner, A. P., Flach, B., O'Connell, S., Bock, K. W., Minai, M., Nagata, B. M., Andersen, H., Martinez, D. R., Noe, A. T., Douek, N., Donaldson, M. M., Nji, N. N., Alvarado, G. S., Edwards, D. K., … Graham, B. S. (2020). Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. *New England Journal of Medicine*, *383*(16), 1544–1555. https://doi.org/10.1056/nejmoa2024671

Creech, C. B., Anderson, E., Berthaud, V., Yildirim, I., Atz, A. M., Melendez Baez, I., Finkelstein, D., Pickrell, P., Kirstein, J., Yut, C., Blair, R., Clifford, R. A., Dunn, M., Campbell, J. D., Montefiori, D. C., Tomassini, J. E., Zhao, X., Deng, W., Zhou, H., … Schnyder Ghamloush, S. (2022). Evaluation of mRNA-1273 Covid-19 Vaccine in Children 6 to 11 Years of Age. *New England Journal of Medicine*, *386*(21), 2011–2023. https://doi.org/10.1056/nejmoa2203315

Cucinotta, D., & Vanelli, M. (2020). WHO declares COVID-19 a pandemic. In *Acta Biomedica* (Vol. 91, Issue 1, pp. 157–160). Mattioli 1885. https://doi.org/10.23750/abm.v91i1.9397

Dagan, N., Barda, N., Kepten, E., Miron, O., Perchik, S., Katz, M. A., Hernán, M. A., Lipsitch, M., Reis, B., & Balicer, R. D. (2021). BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *New England Journal of Medicine*, *384*(15), 1412–1423. https://doi.org/10.1056/nejmoa2101765

Dai, L., Gao, L., Tao, L., Hadinegoro, S. R., Erkin, M., Ying, Z., He, P., Girsang, R. T., Vergara, H., Akram, J., Satari, H. I., Khaliq, T., Sughra, U., Celi, A. P., Li, F., Li, Y., Jiang, Z., Dalimova, D., Tuychiev, J., … Gao, G. F. (2022). Efficacy and Safety of the RBD-Dimer–Based Covid-19 Vaccine ZF2001 in Adults. *New England Journal of Medicine*, *386*(22), 2097–2111. https://doi.org/10.1056/nejmoa2202261

Deplanque, D., & Launay, O. (2021). Efficacy of COVID-19 vaccines: From clinical trials to real life. *Therapies*, *76*(4), 277–283. https://doi.org/10.1016/j.therap.2021.05.004

El Sahly, H. M., Baden, L. R., Essink, B., Montefiori, D., McDermont, A., Rupp, R., Lewis, M., Swaminathan, S., Griffin, C., Fragoso, V., Miller, V. E., Girard, B., Paila, Y. D., Deng, W., Tomassini, J. E., Paris, R., Schödel, F., Das, R., August, A., … Pajon, R. (2022). Humoral Immunogenicity of the mRNA-1273 Vaccine in the Phase 3 Coronavirus Efficacy (COVE) Trial. *Journal of Infectious Diseases*, *226*(10), 1731–1742. https://doi.org/10.1093/infdis/jiac188

Ferdinands, J. M., Rao, S., Dixon, B. E., Mitchell, P. K., Desilva, M. B., Irving, S. A., Lewis, N., Natarajan, K., Stenehjem, E., Grannis, S. J., Han, J., McEvoy, C., Ong, T. C., Naleway, A. L., Reese, S. E., Embi, P. J., Dascomb, K., Klein, N. P., Griggs, E. P., … Fireman, B. (2022). Waning of vaccine effectiveness against moderate and severe covid-19 among adults in the US from the VISION network: Test negative, case-control study. *The BMJ*. https://doi.org/10.1136/bmj-2022-072141

Frenck, R. W., Klein, N. P., Kitchin, N., Gurtman, A., Absalon, J., Lockhart, S., Perez, J. L., Walter, E. B., Senders, S., Bailey, R., Swanson, K. A., Ma, H., Xu, X., Koury, K., Kalina, W. V., Cooper, D., Jennings, T., Brandon, D. M., Thomas, S. J., … Gruber, W. C. (2021). Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. *New England Journal of Medicine*, *385*(3), 239–250. https://doi.org/10.1056/nejmoa2107456

Heath, P. T., Galiza, E. P., Baxter, D. N., Boffito, M., Browne, D., Burns, F., Chadwick, D. R., Clark, R., Cosgrove, C., Galloway, J., Goodman, A. L., Heer, A., Higham, A., Iyengar, S., Jamal, A., Jeanes, C., Kalra, P. A., Kyriakidou, C., McAuley, D. F., … Toback, S. (2021). Safety and Efficacy of NVX-CoV2373 Covid-19 Vaccine. *New England Journal of Medicine*, *385*(13), 1172–1183. https://doi.org/10.1056/nejmoa2107659

Jackson, L. A., Anderson, E. J., Rouphael, N. G., Roberts, P. C., Makhene, M., Coler, R. N., McCullough, M. P., Chappell, J. D., Denison, M. R., Stevens, L. J., Pruijssers, A. J., McDermott, A., Flach, B., Doria-Rose, N. A., Corbett, K. S., Morabito, K. M., O'Dell, S., Schmidt, S. D., Swanson, P. A., … Beigel, J. H. (2020). An mRNA Vaccine against SARS-CoV-2 — Preliminary Report. *New England Journal of Medicine*, *383*(20), 1920–1931. https://doi.org/10.1056/nejmoa2022483

Jiao, K. (2022). A brief introduction and evaluation of typical mRNA vaccine, inactivated vaccine, vectored vaccine, sub-unit vaccine for COVID protection. *Advances in Social Science, Education and Humanities Research, 664*, 279–285. https://doi.org/10.2991/assehr.k.220504.052

Khobragade, A., Bhate, S., Ramaiah, V., Deshpande, S., Giri, K., Phophle, H., Supe, P., Godara, I., Revanna, R., Nagarkar, R., Sanmukhani, J., Dey, A., Chozhavel Rajanathan, T. M., Kansagra, K., & Koradia, P. (2022). Efficacy, safety, and immunogenicity of the DNA SARS-CoV-2 vaccine (ZyCoV-D): The interim efficacy results of a phase 3, randomised, double-blind, placebo-controlled study in India. *The Lancet, 399*(10332), 1313–1321. https://doi.org/10.1016/S0140-6736(22)00151-9

Logunov, D. Y., Dolzhikova, I. V., Shcheblyakov, D. V., Tukhvatulin, A. I., Zubkova, O. V., Dzharullaeva, A. S., Kovyrshina, A. V., Lubenets, N. L., Grousova, D. M., Erokhova, A. S., Botikov, A. G., Izhaeva, F. M., Popova, O., Ozharovskaya, T. A., Esmagambetov, I. B., Favorskaya, I. A., Zrelkin, D. I., Voronina, D. V., Shcherbinin, D. N., … Gintsburg, A. L. (2021). Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *The Lancet*, *397*(10275), 671–681. https://doi.org/10.1016/S0140-6736(21)00234-8

Moreira, E. D., Kitchin, N., Xu, X., Dychter, S. S., Lockhart, S., Gurtman, A., Perez, J. L., Zerbini, C., Dever, M. E., Jennings, T. W., Brandon, D. M., Cannon, K. D., Koren, M. J., Denham, D. S., Berhe, M., Fitz-Patrick, D., Hammitt, L. L., Klein, N. P., Nell, H., … Jansen, K. U. (2022). Safety and Efficacy of a Third Dose of BNT162b2 Covid-19 Vaccine. *New England Journal of Medicine*, *386*(20), 1910–1921. https://doi.org/10.1056/nejmoa2200674

Msemburi, W., Karlinsky, A., Knutson, V., Aleshin-Guendel, S., Chatterji, S., & Wakefield, J. (2023). The WHO estimates of excess mortality associated with the COVID-19 pandemic. *Nature*, *613*(7942), 130–137. https://doi.org/10.1038/s41586-022-05522-2

Mulligan, M. J., Lyke, K. E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Raabe, V., Bailey, R., Swanson, K. A., Li, P., Koury, K., Kalina, W., Cooper, D., Fontes-Garfias, C., Shi, P. Y., Türeci, Ö., Tompkins, K. R., Walsh, E. E., … Jansen, K. U. (2020). Phase I/II study of COVID-

19 RNA vaccine BNT162b1 in adults. *Nature*, *586*(7830), 589–593. https://doi.org/10.1038/s41586-020-2639-4

Noor, R. (2021). *Developmental Status of the Potential Vaccines for the Mitigation of the COVID-19 Pandemic and a Focus on the Effectiveness of the Pfizer-BioNTech and Moderna mRNA Vaccines*. https://doi.org/10.1007/s40588-021-00162-y/Published

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines-a new era in vaccinology. In *Nature Reviews Drug Discovery* (Vol. 17, Issue 4, pp. 261–279). Nature Publishing Group. https://doi.org/10.1038/nrd.2017.243

Park, J. W., Lagniton, P. N. P., Liu, Y., & Xu, R. H. (2021). Mrna vaccines for covid-19: What, why and how. In *International Journal of Biological Sciences* (Vol. 17, Issue 6, pp. 1446–1460). Ivyspring International Publisher. https://doi.org/10.7150/ijbs.59233

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J. L., Pérez Marc, G., Moreira, E. D., Zerbini, C., Bailey, R., Swanson, K. A., Roychoudhury, S., Koury, K., Li, P., Kalina, W. V., Cooper, D., Frenck, R. W., Hammitt, L. L., … Gruber, W. C. (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *New England Journal of Medicine*, *383*(27), 2603–2615. https://doi.org/10.1056/nejmoa2034577

Rodríguez-Chávez, S., Reyes-Zamora, M. C., Castro-Almarales, R. L., Vega-Valdés, J. C., Díaz-Barroso, Y., Rodríguez-Barrientos, D., & Izquierdo-Alfonso, N. (2023). Safety of SOBERANAS® vaccines in children of workers of a BioCubaFarma institution. *VacciMonitor*, *32*. https://doi.org/10.1056/NEJMoa2116298

Saadh, M. J., & Jaber, S. A. (2022). Efficacy of COVID-19 vaccines. *Microbial Pathogenesis*, *171*. https://doi.org/10.1016/j.micpath.2022.105729

Saddik, B., Elbarazi, I., Temsah, M. H., Saheb Sharif-Askari, F., Kheder, W., Hussein, A., Najim, H., Bendardaf, R., Hamid, Q., & Halwani, R. (2021). Psychological Distress and Anxiety Levels Among Health Care Workers at the Height of the COVID-19 Pandemic in the United Arab Emirates. *International Journal of Public Health*, *66*. https://doi.org/10.3389/ijph.2021.1604369

Sadoff, J., Gray, G., Vandebosch, A., Cárdenas, V., Shukarev, G., Grinsztejn, B., Goepfert, P. A., Truyers, C., Fennema, H., Spiessens, B., Offergeld, K., Scheper, G., Taylor, K. L., Robb, M. L., Treanor, J., Barouch, D. H., Stoddard, J., Ryser, M. F., Marovich, M. A., … Douoguih, M. (2021). Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *New England Journal of Medicine*, *384*(23), 2187–2201. https://doi.org/10.1056/nejmoa2101544

Sahin, U., Muik, A., Derhovanessian, E., Vogler, I., Kranz, L. M., Vormehr, M., Baum, A., Pascal, K., Quandt, J., Maurus, D., Brachtendorf, S., Lörks, V., Sikorski, J., Hilker, R., Becker, D., Eller, A. K., Grützner, J., Boesler, C., Rosenbaum, C., … Türeci, Ö. (2020). COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature*, *586*(7830), 594–599. https://doi.org/10.1038/s41586-020-2814-7

Starostina, E. V., Sharabrin, S. V., Antropov, D. N., Stepanov, G. A., Shevelev, G. Y., Lemza, A. E., Rudometov, A. P., Borgoyakova, M. B., Rudometova, N. B., Marchenko, V. Y., Danilchenko, N. V., Chikaev, A. N., Bazhan, S. I., Ilyichev, A. A., & Karpenko, L. I. (2021). Construction and immunogenicity of modified mrna-vaccine variants encoding influenza virus antigens. *Vaccines*, *9*(5). https://doi.org/10.3390/vaccines9050452

Tanriover, M. D., Doğanay, H. L., Akova, M., Güner, H. R., Azap, A., Akhan, S., Köse, Ş., Erdinç, F. Ş., Akalın, E. H., Tabak, Ö. F., Pullukçu, H., Batum, Ö., Şimşek Yavuz, S., Turhan, Ö., Yıldırmak, M. T., Köksal, İ., Taşova, Y., Korten, V., Yılmaz, G., … Aksu, K. (2021). Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *The Lancet*, *398*(10296), 213–222. https://doi.org/10.1016/S0140-6736(21)01429-X

Thomas, S. J., Moreira, E. D., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J. L.,

Pérez Marc, G., Polack, F. P., Zerbini, C., Bailey, R., Swanson, K. A., Xu, X., Roychoudhury, S., Koury, K., Bouguermouh, S., Kalina, W. V., Cooper, D., Frenck, R. W., … Jansen, K. U. (2021). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. *New England Journal of Medicine*, *385*(19), 1761–1773. https://doi.org/10.1056/nejmoa2110345

Udwadia, Z. F., Singh, P., Barkate, H., Patil, S., Rangwala, S., Pendse, A., Kadam, J., Wu, W., Caracta, C. F., & Tandon, M. (2021). Efficacy and safety of favipiravir, an oral RNA-dependent RNA polymerase inhibitor, in mild-to-moderate COVID-19: A randomized, comparative, open-label, multicenter, phase 3 clinical trial. *International Journal of Infectious Diseases*, *103*, 62–71. https://doi.org/10.1016/j.ijid.2020.11.142

Verbeke, R., Lentacker, I., De Smedt, S. C., & Dewitte, H. (2021). The dawn of mRNA vaccines: The COVID-19 case. *Journal of Controlled Release*, *333*, 511–520. https://doi.org/10.1016/j.jconrel.2021.03.043

Vogel, A. B., Kanevsky, I., Che, Y., Swanson, K. A., Muik, A., Vormehr, M., Kranz, L. M., Walzer, K. C., Hein, S., Güler, A., Loschko, J., Maddur, M. S., Ota-Setlik, A., Tompkins, K., Cole, J., Lui, B. G., Ziegenhals, T., Plaschke, A., Eisel, D., … Sahin, U. (2021). BNT162b vaccines protect rhesus macaques from SARS-CoV-2. *Nature*, *592*(7853), 283–289. https://doi.org/10.1038/s41586-021-03275-y

Voysey, M., Clemens, S. A. C., Madhi, S. A., Weckx, L. Y., Folegatti, P. M., Aley, P. K., Angus, B., Baillie, V. L., Barnabas, S. L., Bhorat, Q. E., Bibi, S., Briner, C., Cicconi, P., Collins, A. M., Colin-Jones, R., Cutland, C. L., Darton, T. C., Dheda, K., Duncan, C. J. A., … Pollard, A. J. (2021). Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet*, *397*(10269), 99–111. https://doi.org/10.1016/S0140-6736(20)32661-1

World Health Organization. (2020, March 11). *WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020*. World Health Organization. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

World Health Organization. (2021). *Coronavirus disease (COVID-19) pandemic*. World Health Organization. https://www.who.int/emergencies/diseases/novel-coronavirus-2019

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science, 367*(6483), 1260–1263. https://doi.org/10.1126/science.abb2507