**Demystifying mRNA vaccines: an emerging platform at the forefront of cryptic diseases**

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**ABSTRACT**

Messenger RNA (mRNA) vaccines have been studied for decades, but only recently, during the COVID-19 pandemic, has the technology garnered noteworthy attention. In contrast to traditional vaccines, mRNA vaccines elicit a more balanced immune response, triggering both humoral and cellular components of the adaptive immune system. However, some inherent hurdles associated with stability, immunogenicity, in vivo delivery, along with the novelty of the technology, have generated scepticism in the adoption of mRNA vaccines. Recent developments have pushed to bypass these issues and the approval of mRNA-based vaccines to combat COVID-19 has further highlighted the feasibility, safety, efficacy, and rapid development potential of this platform, thereby pushing it to the forefront of emerging therapeutics. This review aims to demystify mRNA vaccines, delineating the evolution of the technology which has emerged as a timely solution to COVID-19 and exploring the immense potential it offers as a prophylactic option for other cryptic diseases.

**Introduction**

The concept of vaccination developed in the 18th century, and since the first vaccine, the life expectancy of the human population has increased while mortality rates from infectious agents have diminished. Initially, animal viruses were used as vaccines, but the resulting virulence led to the use of microbes that had been reassorted or those that had the pathogenic components inactivated. Subsequent improvements involved attempting to utilise surface glycoproteins or proteins of the non-virulent structures as vaccines [1]. Although each of these systems generally conferred immunity against the targeted pathogens, the need for more simplified and safer methods paved the way toward using a gene-based approach to design vaccines.

The idea of genetic transfer materialised in the 1900s by a series of findings including genetic material transfer between bacterial strains, viral DNA transfer into bacteria, and the ability to rescue genetic defects via transfer of functional DNA from a foreign source [2–4]. The application of gene therapy to treat various genetic disorders over the following decades further piqued interest in the use of genetic material in vaccines.

Since the 1980s, modifications to nucleic acids have been developed towards therapy and vaccine design [5]. While DNA was the first genetic material to be engineered for this purpose, the notion of using the downstream mRNA transcript for more efficient targeting soon followed with gradual developments in mRNA technology, as highlighted in Figure 1 [6–19]. The prospect of mRNA in therapeutics was initially highlighted in 1989 with the demonstration that mRNA, within a cationic lipid, can be directly delivered and expressed in eukaryotic cells for manipulation of gene expression. Subsequently, the initial basis for mRNA as a vaccine platform was established when naked in vitro-transcribed (IVT) mRNA was directly injected into mouse skeletal muscles, and effective expression was observed in vivo [8]. The potential to use exogenous mRNA as a vaccine was further highlighted by the ability of mRNA to induce an antigen-specific immune response. This was evidenced by the presence of virus-specific cytotoxic T lymphocytes following injection of liposome-enclosed mRNA for influenza virus nucleoprotein [9]. In addition, mRNA encoding a carcinoembryonic antigen led to antitumoural antibody responses [20]. Overall, these findings indicated the ability of mRNA vaccines to elicit both cellular and humoral immunity in the host, making mRNA a potential vaccine candidate for infectious diseases and cancers [21].

In recent times, mRNA vaccines have been widely researched for the treatment of a diverse array of diseases.
Some effective mRNA vaccines have been developed for various cancers (e.g. breast and lung cancer) and one such example is BNT111 which has proven effective in clinical trials for the treatment of advanced melanoma [22]. There are also several potential mRNA vaccines for combating infectious diseases by targeting the respective causative agents (e.g. Zika virus, cytomegalovirus, influenza virus, metapneumovirus etc.). One such candidate is mRNA-1893 which is currently under clinical trial for the treatment of Zika virus infections [23]. However, the rapid emergence of mRNA vaccines has been substantially triggered by the COVID-19 pandemic. In a process that generally involves several years of testing for efficacy and safety of a prospective therapeutic, mRNA vaccines have emerged within a landmark timeframe of one year since the start of COVID-19.

In line with this renewed global interest in mRNA vaccines, this review discusses the evolution of mRNA vaccine technology, highlighting key principles, obstacles, solutions, and dissecting crucial factors that determine the acceptability of this vaccine regime. Within its scope, the review targets to resolve the apparent unfamiliarity regarding mRNA vaccines as it discusses the current approaches and future possibilities of its potential to prevent and treat obscure diseases.

**The concept of mRNA vaccines**

The key to mRNA vaccines gradually transitioning into one of the most versatile platforms for prophylaxis lies within their structure and mechanism of action. This transcension can be best justified with a deeper dive into the limitations of alternative formats of vaccines.

**From traditional to mRNA vaccines: the good and the bad**

Traditional vaccines are typically based on the live attenuation of a pathogen to elicit an immune response in the host cells. While this is usually effective in generating a strong immune response, there is a possibility that the live-attenuated pathogen may revert to a virulent form, thereby leaving the host severely at risk of contracting an infection, especially in the case of immunocompromised individuals (e.g. HIV patients, pregnant women, individuals undergoing chemotherapy) [24]. Moreover, the production and engineering process for live-attenuated vaccines is expensive and time-consuming, historically having taken even up to a decade for development [25].

To circumvent these issues surrounding whole inactivated cells, subunit vaccines, which involve only specific parts of the pathogen, may deliver a more targeted approach. However, subunit vaccines usually require the support of adjuvants to stimulate an adequate immune response, since they lack the efficacy derived from live-attenuated vaccines [23,26]. In addition to their respective limitations, a common problem with both live-attenuated and subunit vaccines involves their inability to induce CD8-mediated cellular immunity which may render such vaccines ineffective in combating
intracellular viral infections or tumours [27]. Moreover, developing these vaccines involves working with live pathogens in cell cultures which poses a health and safety complexity.

Therefore, vaccines based on plasmid DNA (pDNA) or mRNA constructs emerged to traverse many of the limitations of traditional vaccines. DNA and mRNA vaccines, being directly based on genetic constructs of the desired antigen of a target pathogen, allow for faster manufacturing, making them ideal for use in case of emerging diseases and urgent scenarios like the COVID-19 pandemic. Additionally, mRNA and DNA vectors are both intrinsically immunostimulatory, capable of modification to produce self-amplifying molecules, and can effectively induce a balanced immune response that involves both the humoral and cell-mediated components of the immune system in contrast to traditional vaccines [24,28]. DNA vaccines have been favoured generally because DNA constructs are more stable in vivo compared to mRNA and have existed longer as a vaccine technology. However, using pDNA can be detrimental due to factors such as uncontrolled long-term expression and conferring antibiotic resistance from the presence of additional foreign genes [29].

In comparison, mRNA, which is only transiently expressed, serves as a better platform since it eliminates the possibility of integration within the host genome and consequent insertional mutagenesis [23]. This transient nature of mRNA also promotes the production of higher affinity antibodies, as the mRNA-encoded antigen becomes scarcer over time. Consequently, a booster dose of the mRNA can lead to the widespread availability of these high-affinity antibodies. Another advantage that mRNA vaccines present over DNA vaccines in terms of delivery stems from the difference in the site of action of these constructs. While the DNA delivered to the cells must cross the nuclear membrane to be transcribed and its downstream mRNA transported to the cytosol to be translated, mRNA vaccines eliminate the first step of this process, simply needing to reach the cytosol to be translated [25].

**Design and mechanism of action of mRNA vaccines**

At the core of mRNA vaccines is an mRNA sequence designed to transiently express the antigen of interest, mimicking a key protein of the pathogen targeted for eradication. In their most basic form, mRNA vaccines contain an open reading frame (ORF) for the target antigen flanked by untranslated regions (UTRs), an N-glycosine cap (m’G/Cap0) at the 5’-end, and a 3’-end terminal poly(A) tail. Following *in vivo* entry and triggering a local immunological response at the site of infection, the antigen of interest is generated by translation and presented as peptides on the transfected antigen-presenting cells (APCs). Consequently, a dual-pronged response, consisting of both innate and adaptive immune pathways, is stimulated comprising of humoral and cellular components, as elucidated in Figure 2 [21,30,31].

The innate immune response is initiated by the recognition of non-self RNA, either cytosolic or endosomal, by pathogen-associated molecular pattern (PAMP) recognition. While toll-like receptors (TLRs) sense endosomal RNA, pattern recognition receptors (PRRs) such as melanoma differentiation-associated 5 (MDA-5), nucleotide oligomerisation domain 2 (NOD2), and retinoic acid-inducible gene I (RIG-I) sense cytosolic RNA. TLR7/8 trigger the myeloid differentiation marker 88 (MyD88) pathway, successively priming B cell activation against the internalised mRNA while TLR3 leads to the production of interferon-beta (IFN-β). Furthermore, TLR7 enhances antigen presentation, promotes inflammatory cytokine secretion and enhances memory B cell survival. The cascade crescendoes with the release of type I interferon (IFN-I) which is instrumental in establishing an antiviral response by the release of proinflammatory cytokines, T-helper 1 (Th1) pathway activation, and APC activation [30].

On the other hand, the adaptive immune response requires the manifestation of the antigen translated from the mRNA. This antigen has to be presented on major histocompatibility complexes (MHCs) on APCs after degradation by intracellular proteasomes. If the antigen epitopes are presented on MHC I complex, a cytotoxic cellular response is initiated when the antigen interacts with CD8+ T lymphocytes. Conversely, exogenously secreted antigens can be endocytosed, degraded by other circulating APCs and consequently presented on MHC II complexes, fostering CD4+ T-helper lymphocyte- and B lymphocyte-mediated response. The combined interaction potentiates the production of high-affinity antibodies specific to the antigen and thereby the pathogen alongside the development of a memory B cell repertoire stowed away until the next infection by this pathogen arises [21].

mRNA vaccines can be categorised into two groups: conventional mRNA vaccines and self-amplifying mRNA (SAM) vaccines. While conventional mRNA vaccines are designed to code for only the antigen of interest, SAM contains an additional ORF encoding a viral RNA-dependent RNA polymerase, which in turn amplifies the production of the mRNA of interest, thereby significantly enhancing the potency of the vaccine at a much lower dose [32].

**Synthesis pipeline of mRNA vaccines**

The fast-track development of mRNA vaccines compared to other platforms is due to the ability to synthesise IVT-mRNA in a cell-free environment, as illustrated in Figure 3 [33,34]. The mRNA developed by this process mimics endogenous mRNA to the extent that it can undergo translation and regulation by the intracellular machinery to synthesise the antigen of interest. Following synthesis and purification, the mRNA transcript is introduced within a delivery vehicle to be injected intramuscularly. To deliver synthetic mRNA *in vivo*, an array of exploratory options exists, ranging from encapsulation within non-viral vectors (e.g. polymer or lipid nanoparticles) to utilising modified retroviral particles (e.g. foamy virus particles) to facilitate efficient transfection within the host [35].

**Therapeutic evolution of mRNA vaccines**

Despite the novel mechanisms and promising therapeutic avenues offered by mRNA vaccines, the adaptation of this
system as a commercially mainstream vaccine platform has been hindered by several obstacles. First, being a relatively large molecule (10^4–10^6 Daltons) with a net negative charge, exogenous mRNA faces electrostatic repulsion from negatively charged carbohydrate side chains on membrane proteins, thereby leading to inefficient permeation across the membrane [23,36]. Therefore, specialised delivery systems are required for efficient in vivo delivery of mRNA. Second, native mRNA is inherently unstable with a half-life of a few hours due to its high susceptibility to degradation by nucleases, and this necessitates structural modifications to mRNA to minimise degradation [37,38]. Third, exogenous mRNA may have immunogenic properties due to its ability to act as a self-adjuvant, ultimately triggering the secretion of inflammatory cytokines and T-cell activation [36,39]. Although this immunogenic property of mRNA boosts its efficacy as a vaccine, an unchecked immune response may lead to mRNA degradation and cause adverse effects. Therefore, strategies to achieve a balanced immune response toward mRNA vaccines are required. To circumvent some of these hurdles, the past decade has witnessed substantial breakthroughs, focusing on the structure and delivery of mRNA, some of which are summarised in Figure 1.

5’ capping
An evolutionarily conserved hallmark of eukaryotic mRNAs, capping at the free 5’-end of mRNA transcripts with m’G/ Cap0 plays a crucial role in cap-dependent translation initiation, RNA splicing, and intracellular transport of mRNA [40]. Similarly, IVT-mRNA requires 5’ capping for stability and robust translation efficiency.

Figure 2. Activation of the immune system by mRNA vaccines. (1) The innate immune response is triggered via pathogen-associated molecular pattern (PAMP) recognition on non-self RNA. This recognition is mediated by pattern recognition receptors (PRRs) such as melanoma differentiation-associated 5 (MDA-5), nucleotide oligomerisation domain 2 (NOD2), and retinoic acid-inducible gene 1 (RIG-I) or toll-like receptors (TLRs), which ultimately results in type I interferon (IFN-I) release, allowing upregulation of proinflammatory genes, activation of the Th1 pathway and APC activation. (2) The internalised mRNA can be translated to generate the antigen, which is presented on the major histocompatibility complexes (MHC) on the APCs, initiating the adaptive response. (3) The antigen degraded by proteasomes is presented as peptides on the MHC I complex, leading to interactions with CD8+ cytotoxic T lymphocytes and triggering their maturation for a cellular response (indicated by blue arrows). (4) Additionally, antigens secreted exogenously can be internalised by other APCs, degraded into peptides within lysosomal endosomes, and presented on MHC II complexes on the cell surface (indicated by green arrows). This stimulates CD4+ T-helper cell interactions, which prime B lymphocytes on maturation for an antibody-mediated humoral response and generate memory cells specific to the target pathogen.
However, in *in vitro* transcription, m\textsuperscript{7}G/Cap0 competes with GTP for incorporation into the mRNA, resulting in inactive and/or uncapped IVT-mRNA [41,42]. Although the cap analogue m\textsuperscript{7}GpppG was initially used to counteract this issue, this resulted in the formation of mRNAs with a mixture of cap analogues in the correct and reverse directions, leading to sub-standard IVT-mRNA synthesis and poor translation efficiency [41]. Since then, translation efficiency and half-life have been substantially increased by introducing an anti-reverse cap analogue (ARCA, m\textsuperscript{7,5}GpppG), where replacing the 3'-OH of the m\textsuperscript{7}G moiety with a 3'-O-methyl group forces ARCA incorporation in the correct orientation and escape from the mRNA-decaping enzyme, Dcp2 [43].

Additional methylation at the 2'-OH of the first nucleotide generates Cap1 (m\textsuperscript{7}GpppNm) [44]. Compared to Cap0, Cap1 reduces immunogenicity by eluding the RNA sensor, retinoic acid-inducible gene I (RIG-I) and RNA binding proteins, such as IFN-induced proteins with tetraticlopeptide repeats (IFIT 1/5) [42]. Another co-transcriptional capping system called CleanCap exploits the capped trimer which results in a naturally occurring Cap1 structure without altering immunogenic response. CleanCap additionally may increase the efficiency of *Cas9* mRNA [7].

### 3' poly(A) tail modification

The 3' poly(A) tail is another feature of eukaryotic mRNAs that has a crucial role in protecting mRNA from enzymatic degradation which conclusively increases stability and extends gene expression [30]. Together with the 5' cap, it forms a ribonucleoprotein (RNP) complex that regulates translation.

The length of the 3' poly(A) tail influences the stability of an IVT-mRNA. It has been reported that the appropriate length of the 3' poly(A) tail for higher translation efficiency varies between cell types and, therefore, needs optimisation before being employed in the design of mRNA vaccines for clinical use [45].
In IVT-mRNA, a 3’ poly(A) tail can be incorporated by encoding a stretch of adenine nucleotides into the template DNA or by employing a two-step enzymatic reaction using a poly(A) polymerase during transcription [46]. However, enzymatic polyadenylation has the disadvantage of generating a 3’ poly(A) tail with an undefined length in each IVT-mRNA preparation. On the contrary, mRNA synthesis from template DNA results in a predetermined length of the 3’ poly(A) tail which is ideal for its utilisation in clinical purposes [14].

**Optimising 5’ and 3’ untranslated regions**

Untranslated regions (UTRs) are the noncoding sequences located upstream and downstream of the ORF in an mRNA denoted as 5’ and 3’ UTRs, respectively. Physiologically, UTRs play a critical role in mRNA stability and translation efficiency by interacting with RNA-binding proteins [30,33].

During the design of mRNA vaccines, it is imperative to optimise these UTRs for better stability and translation efficiency.

The 5’ UTR, which influences the translation of the downstream coding region, can be optimised in several ways. By ensuring that the 5’ UTR has a sequence that is not similar to the ORF sequence, undesirable disruptions to the translation of mRNA can be avoided [47]. It has been reported that inserting GCC-(A/G)-CCAUGG sequence in the 5’ UTR results in a more accurate initiation of mRNA translation [48].

A short 5’ UTR has also been suggested to enhance translation efficiency in mRNAs where highly stable secondary structures obstruct translation [49]. It has also been reported that reduced secondary structures in the 5’ UTR and the first ~30 codons of the CDS results in enhanced gene expression [45].

On the other hand, the 3’ UTR regulates mRNA stability and translation efficiency. As the 3’ UTR is notorious for its unstable regulatory elements, more stable α- and β-globin 3’ UTRs are widely used in IVT-mRNA to circumvent this problem [50,51]. It has been reported that the incorporation of two human β-globin 3’ UTRs in a head-to-tail orientation can enhance the stability of mRNAs [50]. Alternatively, AU and GU rich sequences can be used to further improve the stability of mRNAs [52,53]. Unexpectedly, increased secondary structures in the CDS (after the first ~30 codons) and 3’ UTR are associated with higher translation efficiency [45].

**Base modification**

Chemical modification of nucleoside base(s) in mRNA plays an important role in enhancing gene expression and significantly reducing immunogenicity. However, among 172 base modifications [54], only a few have been reported to facilitate gene expression, reduce immunogenicity, and increase stability. One example is the methylation of cytosine to 5-methylcytosine (mC) in GC-rich regions of RNA, catalysed by N(2)O2/Sun RNA methyltransferase 2. The recognition of 5-methylcytosine by the mRNA export adaptor protein, Aly/REF export factor, results in enhanced gene expression [55].

Another base modification involves pseudouridylation, where pseudouridine synthase catalyses the site-specific isomerisation of uridine into pseudouridine (ψ) during post-transcriptional modification of RNA [56]. Through RNA-RNA and RNA-protein interactions [57], pseudouridine increases gene expression and reduces immunogenicity [58][59].

Another uridine base modification, 2-thiouridine (s2U), facilitates increased binding affinity through Watson-Crick base pairing with adenosine and wobble pairing with guanosine at the third codon [60], enhancing downstream translation efficiency [61]. 5-methylcytosine, pseudouridine, and 2-thiouridine when implemented in synthetic mRNA, enable the mRNA to escape detection by the innate immune system by significantly reducing TLR3/7/8 and RIG-I activation [42].

The second generation of uridine modifications includes N1-methylpseudouridine (m1ψ), which is the most frequently incorporated base modification in mRNA for therapeutic purposes. This is due to its robust ability to increase translation efficiency by promoting dephosphorylation of eukaryotic initiation factor 2 alpha (eIF2α) and to reduce immunogenicity compared to previous generations of uridine modifications [62,63]. m1ψ has been also reported to increase base pair stability resulting in complex secondary structures which enhance translation efficiency [45].

The most common modification in eukaryotic RNA, N6-methyladenosine (m6A), is important for a range of processes from RNA splicing to regulation of gene expression [64]. However, its specific role in IVT-mRNA is still obscure and may be related to increased stability [65].

More recently, N4-acetylcystidine (ac4C), a new class of RNA modification catalysed by N-acetyltransferase 10, has been reported to improve translational yield and mRNA stability [18].

Among other modifications, the site-specific role of 5-methoxyuridine (5moU) is associated with decreased immunogenicity [7]. Hence, undesirable immunogenicity can be diminished by using a range of naturally occurring nucleoside modifications and modulating expression kinetics while monitoring immunogenicity in real-time with RNA immunogenic assay [66].

**Engineering open reading frames**

There are 61 specialised tRNAs to decode codons required for protein synthesis. However, because of wobble base pairing, not all the tRNAs are required to successfully translate mRNAs. Harnessing this codon redundancy, ORF sequences can be engineered to increase translation efficiency by replacing the codon of a target sequence without altering the resulting amino acid composition. This can be achieved by incorporating synonymous frequent codons and/or codons with increased tRNA abundance to replace codons with rare tRNAs [67].

It has also been reported that ORFs with a higher GC content result in a 100-fold higher translation rate compared to ORFs with a low GC content [68]. An optimised ORF in IVT-mRNA further enhances translation efficiency by escaping RNA sensors (RIG-I, TLR3/7/8 and MDA5) [7]. The utilisation of sequence-engineered unmodified and pseudouridine triphosphate-modified mRNA robustly enhances...
translation efficiency [17,69]. Intriguingly, some proteins require a delayed translation rate to undergo proper folding and this can be readily accomplished by employing rare codons in the ORF [30].

**High-temperature IVT-mRNA synthesis**

One of the major problems associated with IVT-mRNA synthesis is immunogenicity caused by double-stranded RNA (dsRNA). The conventional IVT-mRNA synthesis process includes the use of phage RNA polymerases (RNAPs) which bears the burden of generating dsRNA as a by-product, predominantly through the run-off product of 3’ extension and the production of antisense RNAs [70].

Recently, utilisation of thermostable T7 RNAPs have been reported to minimise the production of 3’ extension of run-off products and are functional in vivo with reduced immunogenicity. Furthermore, combining template encoding a long poly(A) tail with thermostable T7 RNAPs prevent both kinds of by-product formation [70]. Therefore, high-temperature IVT-mRNA synthesis can help bypass post-synthesis purification, making mRNA vaccine production more economically feasible.

**Circularisation of mRNA**

To counteract problems associated with the short half-life of mRNA, circularisation of the linear mRNA may present as a promising solution. Circular RNAs do not have the free ends that are present in linear RNA, making them resistant to degradation by nucleases, thereby enhancing overall stability and half-life [71].

Protein-coding mRNAs of interest can be circularised via the inclusion of self-splicing introns during mRNA design in vitro. Circularisation has been shown to enhance the stability and translation efficiency of the full-length mRNA in vitro in eukaryotic cells [72]. The efficacy of circular RNA has also been demonstrated in vivo in mice, where it was efficiently translated without adversely stimulating the immune system. Furthermore, the protein expressed from circular RNA was more stable compared to that expressed by linear mRNA [73].

**Modulation of mRNA expression with riboswitch**

Riboswitch is a regulatory element usually located in the 5’ UTR of some mRNAs and it regulates the magnitude of gene expression. In the past, extensive efforts were invested towards exploiting this crude technology to control gene expression at the mRNA level [74–78].

Only recently, it has been reported that the expression of synthetic mRNA can be controlled both in vitro and in vivo with a kill switch by adding the US Food and Drug Administration (FDA)-approved drug, trimethoprim (TMP) [75,79]. In this mechanism, the designed SAM contains two sub-genomic promoters allowing the initiation of replication of two genes. While the first sub-genomic promoter allows encoding of the TMP-responsive fusion protein destabilising domain L7Ae (DD-L7Ae), the second promoter encodes the therapeutic or reporter protein of interest. As a result, the protein of interest is translated and DD-L7Ae is degraded in the absence of TMP. However, when added, TMP binds to DD-L7Ae which enables the TMP-DD-L7Ae complex to bind to k-turns upstream of the coding sequence of the protein of interest, enabling temporal control of gene expression [75,79]. As such, successful integration of TMP-responsive synthetic mRNA may enhance safety in case of unwanted side effects. This technology may also present an immense potential toward the next generation of therapeutics, vaccines, and diagnostics tools.

**Counteracting immunogenicity with innate inhibiting proteins**

SAM has also been used as a highly efficient vaccine platform for Ebola [80], HIV-1 [81], influenza [82], respiratory syncytial virus (RSV) [83], rabies [84], and COVID-19 [85]. However, unwanted immunogenicity has long been rooted within the mRNA therapy platform. With the activation of the innate immune system, protein kinase R (PKR) and 2’-5’- oligoadenylate synthetase (OAS)/ribonuclease L (RNase L) lead to the degradation and inhibition of mRNA [86].

In addition to the strategies used to minimise immunogenicity related to synthetic mRNA (as discussed in the previous sections), it has recently been reported that innate inhibiting proteins (IIPs) can be incorporated into the mRNA to mitigate the activation of innate immune responses and to enhance gene expression [87]. When parainfluenza virus 5 (PIV-5 V) and Middle East respiratory syndrome coronavirus (MERS-CoV) ORF4a IIPs were encoded in the cis region of the mRNA transcript, protein expression was significantly increased in vitro. MERS-CoV ORF4a also enhanced gene expression in vivo in mice. Importantly, both IIPs reduced immunogenicity by downregulating the activation of interferon regulatory transcription factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). Because self-replication results in an exponential rise in RNA transcripts and dsRNA intermediates in the cytoplasm, offsetting the activation of the innate immune system is critical for SAM therapy [88,89].

**In vivo delivery mechanisms**

*In vivo* delivery of exogenous mRNAs is one of the major challenges in mRNA therapy. Therefore, substantial research has focused on developing more efficient delivery systems. Since a detailed discussion of each delivery platform is beyond the scope of this review, the interested reader is referred to other excellent reviews for more in-depth information on *in vivo* delivery of mRNA [90–92].

Among the available delivery platforms for mRNA therapeutics, lipid nanoparticles (LNPs), which are composed of a complex of synthetic or naturally occurring lipids or lipid-like molecules, are the most widely used. Generally, LNPs consist of ionisable lipids and stabilising molecules such as phospholipids, cholesterol, and polyethylene glycol (PEG). The exogenous mRNA is encapsulated within the lipid shell by electrostatic interactions between the ionisable lipid and
the negatively charged mRNA [90]. LNP-encapsulation has been used for targeted delivery of mRNAs to specific cell types [93] as well as for CRISPR-Cas9-mediated genome editing in vivo in mice [94]. Furthermore, a recent study where LNPs were used to deliver mRNA in utero into mouse foetuses highlights the immense prospect of this platform to aid mRNA therapy in correcting genetic defects before birth [95].

The versatility of LNPs in mediating mRNA delivery is generally due to the ability to tailor the composition of the individual components within the complex. In this regard, modulating the type(s) of the ionisable lipids in the LNP plays a crucial role in the stability and successful intracellular delivery of the mRNA cargo. This has been most prominently demonstrated during the design of LNPs used for the delivery of COVID-19 mRNA vaccines. For example, BNT162b2 vaccine (Pfizer/BioNTech) utilises the cationic lipid ALC-0315 in its carrier LNP whereas mRNA-1273 vaccine (Moderna) utilises a different ionisable lipid, SM-102 [96]. In other examples, LNPs formulated with C1, a cationic lipid-like molecule, have been used to deliver mRNA to dendritic cells leading to efficient antigen presentation to elicit a significant T-cell response. C1 also acted as an adjuvant by inducing an inflammatory cytokine response in dendritic cells. The C1-LNP formulation was also effective at delivering tumour antigen-encoding mRNA in tumour models where a significant anti-tumour response was induced with no cytotoxic effects [97]. In place of cationic lipids, other lipid-like molecules can be used in a subtype of LNP known as lipid-like nanomaterials (LLNs). For example, functionalised TT derivative 5 (FTT5) has been used to deliver human factor VIII mRNA, which is a relatively long mRNA, into haemophilic mice. FTT5-encapsulated mRNA had a slower biodegradability in vivo, thereby persisting longer in the intracellular environment to allow for better mRNA expression without significant cytotoxicity [98].

The inclusion of an adjuvant to stimulate an immune response from mRNA vaccines has also been used in other LNPs. For example, LNP with the adjuvant C16-R848 (a modified TLR7/8 agonist) has been used to enhance mRNA transfection efficiency, antigen presentation, and T-cell response in tumour models, leading to both prevention and suppression of tumour growth [99].

However, in contrast to using adjuvants to boost immune responses, certain circumstances may necessitate the dampening of an excessive immune response. For example, LNPs to deliver human fibroblast growth factor 21 mRNA subcutaneously in vivo in mice have shown immunogenic effects due to the route of administration. Subsequently, the incorporation of hydrocarbon-based ester prodrugs of anti-inflammatory steroids into the LNP alleviated this excessive immune response. Additionally, this enhanced the longevity of protein expression and release into the systemic circulation, highlighting the potential of this system for use in protein replacement therapies. Furthermore, from the patients’ perspective, subcutaneous delivery of mRNA has the additional benefit of therapy self-administration, whereas intravenous administration requires trained personnel [100].

A key limitation with intravenous administration of LNP-encapsulated mRNA is the low bioavailability as a result of scavenging in the liver by the reticuloendothelial system (RES), which is involved with the clearance of toxins [101,102]. To circumvent this, the RES cells can be primed with a nanoprimer before LNP-mRNA injection. Nanoprimer are specifically designed liposomes that, due to their molecular properties, can transiently occupy RES cells, thereby preventing the uptake of LNP-mRNA by these cells. This was efficacious when introduced before LNPs encapsulating human erythropoietin mRNA in mice [103]. In addition to intravenous and subcutaneous routes of administration, aerosolised LNPs have also been shown to facilitate localised delivery of mRNAs into the pulmonary system in vivo in mice [104]. More recently, it has been demonstrated that LNP-encapsulated mRNA may also be delivered orally. In an elegant study, LNP-encapsulated mRNA was further contained in a self-injecting capsule known as ‘self-orienting millimetre-scale applicator (SOMA)’. SOMA capsules, while bypassing degradation by digestive enzymes, were able to inject the LNP-mRNA formulation directly into the gastric lining in vivo in mice and pigs leading to a high transfection efficiency both locally and in the systemic circulation. These findings represent a unique paradigm for mRNA vaccine delivery in circumstances where intravenous or subcutaneous delivery modes may prove difficult [105].

LNPs can be further modulated by the inclusion of branched polymers called dendrimers which contain an inner core and several layers of branched structures, at the exterior of which different functional groups can be incorporated. Drug cargoes can be encapsulated within dendrimers either via covalent or non-covalent interactions [106]. Dendrimer-based lipid nanoparticles (DLNs) are robust in terms of tissue-targeted delivery of mRNA and monitoring of therapy. By incorporating PEGylated fluorescent dyes along with the mRNA within the DNLP, mRNA expression may be enhanced and simultaneously tumours may be visualised upon imaging [107].

Lipid-based vectors smaller than LNPs have also been employed for mRNA packaging and delivery. Nanolipoprotein particles (NLPs), which are analogous to high-density lipoprotein, are discoidal complexes consisting of a lipid bilayer with apolipoproteins. With a smaller yet adjustable diameter, NLPs are more compact compared to LNPs [108,109]. NLPs composed of cationic lipids may have the potential for enhanced in vivo delivery of large self-replicating mRNAs. For example, cationic NLP encasing of mRNA enhanced protection from RNAse degradation and boosted mRNA expression in vivo in mice. By fine-tuning the composition of the cationic lipids and including additional components, such as glycerol monooleate, the translation efficiency of the mRNA could be further improved [110].

Compact packaging of mRNA may also be achieved by bundling mRNA molecules by hybridisation with oligonucleotide crosslinkers and then packaging within PEG-coated polyplex micelles (PMs). This was shown to improve mRNA stability and expression in vivo in mice [111]. Delivery of mRNA using PEG-coated PMs has also been used for CRISPR-Cas9-mediated gene editing in vivo. By co-encapsulating Cas9 mRNA and single-guide RNA (sgRNA), which have drastically different sizes, the stability of both
RNAs as well as genome-editing efficiency in mice significantly improved [112].

Another promising mRNA carrier is the mineral-coated particle (MCM), which has been shown to improve the duration of protein expression in vivo from a single dose of mRNA. The mRNA was transiently translated at high efficiency, and the overexpressed protein was then sequestered within the MCMs to enhance the longevity of the biological response. Furthermore, co-delivering anti-inflammatory proteins with the mRNA using MCMs enhanced transfection efficiency to a level comparable to that of chemically modified mRNA, making this system a viable approach when chemical modification of mRNA is undesirable [113].

A further class of polymers, known as viromers, have also exhibited potential for mRNA delivery. In contrast to LNPs, viromers have a more neutral surface charge and, therefore, do not aggregate in serum. These polymers have been used to successfully deliver therapeutics in mouse models of inflammation [114].

A major drawback to using synthetic lipid carriers such as LNPs for mRNA delivery is the poor ability of these carriers to escape from endosomes upon entry into the cell. As a result, only a portion of the mRNA cargo is delivered to the cytosol. To counteract this issue, recently a delivery system utilising multi-tailed ionisable phospholipids (iPhos) was used to efficiently deliver mRNA for CRISPR-Cas9 gene editing with significantly enhanced endosomal escape. Furthermore, incorporating these ionisable phospholipids with other helper lipids to form hybrid LNPs (iP LNPs) led to mRNA delivery selectively to specific organs in vivo in mice [115].

Apart from using external carriers such as LNPs and other vehicles, a recent study harnessed the potential of retroviral elements within the human genome, thereby using the body's mechanisms as a delivery platform. This was shown using PEG10, a retroviral protein that binds to its mRNA and secretes it extracellularly. By flanking mRNA-encoding sequences with the 5' and 3' UTRs of Peg10, this system termed selective endogenous encapsidation for cellular delivery (SEND), was used as an endogenous vector to deliver mRNAs into mammalian cells [116].

On the whole, a myriad of options exists for in vivo delivery of mRNA with newer strategies emerging at a fascinating pace. In addition, key discoveries centred on the evolution of mRNA technology have generated intellectual property (Box 1). In essence, mRNA vaccines can be optimised at the level of the structural mRNA as well as at the supramolecular assembly, with a vast range of effective strategies at both levels, as indicated in Figure 4. Some of these interventions have recently been incorporated during the design and manufacture of mRNA vaccines targeting COVID-19 and are, therefore, centre-stage to the success of potential vaccines in the future.

**A new era of mRNA vaccines: COVID-19**

The immense potential of mRNA vaccines is best exemplified by the advent of this therapeutic platform in light of the recent COVID-19 pandemic. Although no vaccine for COVID-19, apart from BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna), has obtained full approval by the FDA [120], the World Health Organisation (WHO) has approved some vaccines for emergency use, as listed in Table 1 [121–130]. Some of these vaccines utilise conventional platforms, such as live-attenuated or inactivated viruses (e.g. CoronaVac and BIBP-CorV). However, the possibility of reversion to a pathogenic form in the case of live-attenuated vaccines and the requirement for a multiple-dose regimen to counteract the reduced immune response in the case of inactivated virus vaccines present a challenge. Other COVID-19 vaccines are based on viral vectors, such as the adenovirus vector vaccines (e.g. ChAdOx1 and Ad26.COV2.S), but pre-existing immunity in humans may reduce the efficiency of the adenoviral vector. Therefore, mRNA vaccines have emerged as the most appealing option owing to their safety, production speed, stability, and scalability [131,132]. Currently, 25 different mRNA vaccines against SARS-CoV-2 have advanced to clinical trials [133]. Two of these, manufactured by Pfizer/BioNTech and Moderna, were found to be approximately 95% efficacious in preventing infection.

The design of the COVID-19 mRNA vaccines stems from the attributes of the entry mechanism of the virus into host cells. SARS-CoV-2, the causative agent of COVID-19, attaches to a cell-surface receptor on host cells via a surface-anchored protein called SARS-CoV-2 spike (S) glycoprotein and enters via endocytosis [134–136]. S glycoprotein is found as a trimer in mature viruses with a receptor-binding subunit (S1), a membrane fusion subunit (S2), a transmembrane anchor (TM), and an intracellular tail (IC). The S1 subunit includes a receptor-binding domain (RBD), which binds to angiotensin-converting enzyme 2 (ACE2) on the host cell [137–139]. Upon binding of RBD and ACE2, conformational changes in the S1 and S2 subunits allow virus entry into the cell [140,141]. This conformational change to allow virus attachment is facilitated by cell surface proteases. Inside the host cell, lysosomal protease cathepsins promote the release of the viral genome from the endosome [142,143]. Since the S glycoprotein is necessary for attachment and entry of the virus into the host cells, it is an

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**Box 1 - The patent landscape of mRNA technology**

The first patent family for mRNA-based technology was identified in 1990 with a maturation phase (the time during which the number of patents submitted and granted was relatively low) up to 2010 [117]. Rapid degradation of naked mRNA and poor mRNA delivery systems were mostly associated with the maturation phase. However, with the discovery of modified nucleosides (patent granted to the University of Pennsylvania, USA) the mRNA vaccine field expanded [118]. Subsequently, the utilisation of LNPs to deliver mRNA also played a major role in the growth of the technology (patent granted to the University of British Columbia, Canada) with an increase of approximately nine-fold patent publications from 2009 to 2020 [119]. Through a chain of licence acquisitions, pharmaceuticals such as BioNTech (Mainz, Germany), Moderna (Cambridge, USA), and CureVac (Tübingen, Germany) eventually gained access to the relevant patents enabling the manufacture of COVID-19 mRNA vaccines [118]. CureVac, with more than 800 patents, has the highest number of mRNA-related patents [117]. The recent successful clinical trials of COVID-19 mRNA vaccines have further facilitated an investment surge in companies specialising in mRNA technology as well as in academia, both of which will likely lead to more patent applications in the coming years.
ideal target for vaccines against SARS-CoV-2. Both the approved Pfizer/BioNTech and Moderna COVID-19 vaccines incorporate the S glycoprotein-encoding genetic sequence. Following intramuscular injection of these LNP-encapsulated mRNA vaccines, the mRNA is taken up by host cells and delivered to the cytosol where the mRNA sequence is translated into the S glycoprotein. Following post-translational modification by host cells, the protein is displayed at the cell surface as a membrane-bound antigen in its pre fusion shape, providing an antigen target for B cells towards mounting an antibody response in the host [144].

**BNT162b2 vaccine (Pfizer and BioNTech)**

BNT162b2 mRNA, which encodes the full-length membrane-anchored S glycoprotein of SARS-CoV-2 [145], was co-developed by Pfizer (New York, USA) and BioNTech (Mainz, Germany). Also known as Comirnaty, it was the first vaccine against SARS-CoV-2 to have obtained full approval from the FDA [120].

In phase one trials of this vaccine, participants were administered two vaccinations with doses of either 10 μg, 30 μg, or 100 μg on the first and 22nd days [146]. The 30 μg dose was found to be the most efficacious with minimum safety concerns. The average antibody titres in participants who received the 30 μg dose surpassed that of a human convalescent serum panel obtained from patients who had recently recovered from COVID-19. There was also a high titre of SARS-CoV-2 neutralising antibodies and antigen-specific T-cell responses in the trial participants [147].

Based on this preliminary data from the phase one trials, phase two/three trials further established the efficacy of the vaccine. Whereas 162 participants who received a placebo contracted COVID-19, only eight vaccinated participants
Table 1. A systematic comparison of COVID-19 vaccines.

| Vaccine platform | Delivery system | Dosage | Clinical trial population | T-cell activation | Recommended for ages | Suggested route and site of administration | Efficacy | Effectiveness against new variants of concern | Cost per dose | Preparation | Safety concerns |
|------------------|-----------------|--------|---------------------------|------------------|---------------------|-----------------------------------------|---------|-----------------------------------------------|-------------|-------------|-----------------|
| BNT162b2 (Pfizer/BioNTech) | mRNA-1273 (Moderna) | 30 μg of nucleoside-modified mRNA; 2 doses | Argentina, Brazil, Germany, South Africa, Turkey, and USA: 30,000 participants | Robust expression of T-helper 1 (Th1) and T follicular helper (Thf) type CD4+ responses as well as a robust IFNγ+ IL-2+ CD8+ T-cell responses in adults aged ≥16 years | ≥12 years | Intramuscular, deltoid muscle | 95% | 93.7% against Alpha | Approximately USD 20 | Diluted in 1.8 ml of 0.9% sodium chloride solution before use | Known history of anaphylaxis and immediate allergic reaction (e.g. anaphylaxis, urticaria, angioedema, respiratory distress) to the first dose |
| mRNA-1273 (Oxford/AstraZeneca) | mRNA-1273 (Oxford/AstraZeneca) | 100 μg of nucleoside-modified mRNA; 2 doses | Brazil, South Africa, and UK: 23,745 participants | Robust CD4+ T-cell responses and low CD8+ T-cell responses in adults aged 18–55 years and in older adults ≥65 years | ≥18 years | Intramuscular, deltoid muscle | 94.1% | 100% against Alpha | Approximately USD 20 | Ready to use | Known history of anaphylaxis |
| Ad26.COV2.S (Johnson & Johnson/Janssen) | Adenovirus-based delivery system; adenoviruses are non-enveloped icosahedral particles (virions) | 0.5 ml of 5 × 10^10 AD26.COV2.S recombinant particles; 2 doses | Argentina, Brazil, Chile, Colombia, Mexico, Peru, South Africa, and USA: 43,783 participants | CD4+ and CD8+ T-cell responses by day 15 and up to day 29 in the majority of adults aged 18–55 years and in older adults aged ≥65 years | ≥18 years | Intramuscular, deltoid muscle | 63.1% | 74.5% against Alpha | Below USD 6 (as low as USD 2.15) | Ready to use | Known history of anaphylaxis |
| CoronaVac (Sinovac) | Double-stranded DNA encapsulated by an icosahedral protein structure | 0.5 ml of 5 × 10^9 AD26.COV2.S viral particles; 1 dose | Brazil, Chile, China, Indonesia, and Turkey: 25,000 participants | CD4+ and CD8+ T-cell responses in adults aged 18–59 years and in older adults ≥60 years | ≥18 years | Intramuscular, deltoid muscle | 50.7% | 67% against Delta | USD 10 | Ready to use | Known history of anaphylaxis and capillary leak syndrome |
| BBIBP-CorV (Sinopharm) | -- | 0.5 ml of 3 μg inactivated vaccine; 2 doses | Bahrain, Egypt, Jordan, UAE: 41,301 participants | CD4+ and CD8+ T-cell responses in adults aged 18–59 years and in older adults ≥60 years | ≥18 years | Intramuscular, deltoid muscle | 78.1% | -- | USD 19–36 | Ready to use | Known history of anaphylaxis |

(Continued)
Table 1. (Continued).

| BNT162b2 (Pfizer/BioNTech) | mRNA-1273 (Moderna) | ChAdOx1-S/ AZD1222 (Oxford/Astrazeneca) | Ad26.COV2.S (Johnson & Johnson/Janssen) | CoronaVac (SinoVac) | BBBP-Cov (Sinopharm) |
|---------------------------|---------------------|----------------------------------------|------------------------------------------|---------------------|---------------------|
| **Storage temperature**   |                     |                                        |                                          |                     |                     |
| Undiluted: −90 to −60°C for 6 months from the time of formulation | Frozen, unopened: −25 to −15°C until expiration date | Frozen, unopened: −2°C to −8°C for 6 months | Frozen, unopened: −20°C for 24 months | Unopened: 2–8°C for 12 months or until expiration date | Unopened, pre-filled syringe: 2–8°C for 24 months |
| Undiluted; thawed: 2–8°C for 120 hours before dilution (up to 30°C for 2 hours) | Thawed, unopened: 2–8°C for 30 days (8–25°C for 12 hours) | Thawed, unopened: 2–8°C for 48 hours (up to 30°C for 6 hours) | Thawed, unopened: 2–8°C for 3 months | Thawed, unopened: 2–8°C for 6 hours |                     |
| Diluted: 2–30°C for 6 hours | Thawed, punctured: 2–25°C for 6 hours after withdrawing first dose |                                              |                                          |                     |                     |
| **Light sensitivity**      | Avoid direct exposure to light | Avoid direct exposure to light | Avoid direct exposure to light | Avoid direct exposure to light | Avoid direct exposure to light |
| **Side effects**           | Very common (≥1/10): Injection site swelling, pain, headache, chills, arthralgia, myalgia, and pyrexia (higher frequency after 2nd dose) | Very common (≥1/10): Injection site pain, tenderness, warmth, itching, bruising, fatigue, chills, headache, arthralgia, stiffness, myalgia, and lymphadenopathy | Very common (≥1/10): Injection site pain, headache, nausea, fatigue and myalgia | Very common (≥1/10): Injection site pain, headache, nausea, fatigue and myalgia | Very common (≥1/10): Injection site pain, headache, and fatigue |
| Common (≥1/100 to <1/10): Injection site redness and nausea | Common (≥1/100 to <1/10): Injection site redness or swelling, rash, vomiting, and diarrhea | Common (≥1/100 to <1/10): Injection site redness or swelling, rash, vomiting, and diarrhea | Common (≥1/100 to <1/10): Injection site swelling, redness, and fever (≥38°C) | Very common (≥1/100 to <1/100): Rash, muscle weakness, arm or leg pain, feeling weak and generally unwell, sneezing, sore throat, back pain, tremor, and hyperhidrosis (abnormal sweating) | Common (≥1/100 to <1/100): Rash, muscle weakness, arm or leg pain, feeling weak and generally unwell, sneezing, sore throat, back pain, tremor, and hyperhidrosis (abnormal sweating) |
| Uncommon (≥1/1000 to <1/100): Injection site itching, lymphadenopathy, insomnia, pain in extremities, and malaise | Uncommon (≥1/1000 to <1/100): Injection site itching, lymphadenopathy, insomnia, pain in extremities, and malaise | Uncommon (≥1/1000 to <1/100): Injection site itching, lymphadenopathy, insomnia, pain in extremities, and malaise | Uncommon (≥1/1000 to <1/100): Injection site swelling, redness, and fever (≥38°C) | Rare (≥1/100,000 to <1/1000): Allergic reaction and hives | Rare (≥1/100,000 to <1/1000): Allergic reaction and hives |
| Rare (≥1/10,000 to <1/1000): Bell’s palsy (acute peripheral facial paralysis), mycarditis, and pericarditis | Rare (≥1/10,000 to <1/1000): Bell’s palsy (acute peripheral facial paralysis), mycarditis, and pericarditis | Rare (≥1/10,000 to <1/1000): Facial swelling, Bell’s palsy (acute peripheral facial paralysis), mycarditis, and pericarditis | Very rare (<1/10,000): Neuroinflammatory disorder (transverse myelitis) has been reported but a causal relationship has not been established | Very rare (<1/10,000): Neuroinflammatory disorder (transverse myelitis) has been reported but a causal relationship has not been established | Very rare (<1/10,000): Neuroinflammatory disorder (transverse myelitis) has been reported but a causal relationship has not been established |
* Efficacy defines the measurement of the vaccine’s ability in preventing disease in a controlled and double-blinded clinical trial
** Effectiveness of a vaccine is measured in the real world and counted 14 or more days after the second dose
*** All prices are subject to trademark agreements
- Information not available on peer-reviewed sources
had the disease, indicating a 95% vaccine efficacy. Ultimately, BNT162b2 reached the primary efficacy endpoints, with a greater than 99.99% chance of actual vaccine efficacy greater than 30%. These findings fulfilled the pre-determined requirements, which were to create a chance greater than 98.6% of true vaccine efficacy being greater than 30%, well surpassing FDA’s minimum authorisation criteria [145,148].

In terms of safety, even after the second dose, most participants only experienced mild-to-moderate local reactions which subsided within one to two days. These included pain at the injection site, fatigue, and headache but no long-term side effects. Overall, a two-dose regimen of BNT162b2 provided 95% protection against COVID-19, and for a median of two months, the safety of BNT162b2 was comparable to that of other viral vaccines [145].

**mRNA-1273 vaccine (Moderna and NIAID)**

The mRNA-1273 vaccine was co-developed by the National Institute of Allergy and Infectious Diseases (NIAID, the trial sponsor) and Moderna (Cambridge, USA).

In phase one trial of this vaccine, participants received mRNA-1273 in doses of either 25 μg, 100 μg, or 250 μg on the first and 29th days. Higher doses of the vaccine resulted in a higher antibody titre [149,150]. Those who received 100 μg and 250 μg of the vaccine developed antibodies specific to SARS-CoV-2 within 15 days of the first vaccination. The 100 μg dose was selected for use in further trials since 250 μg of the vaccine caused severe side effects in a few participants [149,151].

In phase three trials of mRNA-1273, 185 participants in the placebo group had onset of symptomatic COVID-19, while only 11 vaccinated participants contracted the disease. Therefore, the efficacy of mRNA-1273 was established as 94.1%. All the extreme COVID-19 cases occurred in the placebo group, implying that mRNA-1273 may have an impact on reducing the severity of the disease, ultimately preventing deaths. Additionally, there were only temporary mild-to-moderate local and systemic reactions (e.g. pain at the injection site, headache, chills, and fatigue) in most participants, thereby highlighting the safety of mRNA-1273 [152]. Overall, a double dose of mRNA-1273 provided 94.1% protection against COVID-19, with only short-term side effects.

More recently, a trial of the mRNA-1273 vaccine was conducted in transplant patients who had already received two doses of the vaccine to assess if a third dose would be safe and effective in improving the immune response in immunocompromised patients. In these individuals, a third dose of the vaccine elicited a significantly stronger immune response compared to the placebo according to analyses of both primary and secondary trial endpoints, thereby indicating the benefits of a booster dose in enhancing immunity [153].

In summary, strong clinical evidence supports the effectiveness of these COVID-19 mRNA vaccines. However, the unfamiliarity within the wider public regarding this new form of vaccination has generated some debate, most of which stems from myths that can be refuted using scientific proof (Box 2). With the growing use of these vaccines, this scepticism is starting to be addressed and it is expected that approval of more mRNA vaccines in the future can elevate this technology to a more widely accepted standard model of vaccination.

**Box 2: Debunking COVID-19 mRNA vaccine myths**

**Myth:** mRNA vaccines can alter the DNA

**Fact:** mRNA vaccines do not enter the nucleus where the DNA resides. Additionally, mRNA itself cannot be incorporated into DNA.

**Myth:** Clinical trials on mRNA vaccines were too fast and the vaccines are unsafe

**Fact:** Three decades of research and several clinical trials have generated substantial data on the safety of mRNA vaccines. In contrast to traditional vaccines, mRNA vaccine formulations can be designed in only a few weeks, making it possible to progress to clinical trials faster. Furthermore, the COVID-19 pandemic created an urgency leading to Pfizer/BioNTech conducting phase 2/3 trials simultaneously to fasten data collection.

**Myth:** COVID-19 mRNA vaccines affect fertility and are unsafe for pregnant women

**Fact:** False media reports created the confusion that the SARS-CoV-2 spike protein is the same as the spike protein of syncytin-1 which plays a crucial role in placental development. Additionally, it was falsely reported that the COVID-19 mRNA vaccines would generate an immune response against syncytin-1. The similarities between SARS-CoV-2 and syncytin-1 spike proteins are only 8.8% and 15.8% for amino acid identity and amino acid sequence respectively. Moreover, there is no cross-reactivity of SARS-CoV-2 and syncytin-1 spike proteins [154]. Thus, there is no high risk associated with COVID-19 vaccination during pregnancy and no adverse effects of the vaccine on fertility [155,156].

**Myth:** Vaccination is not required if one has already had COVID-19

**Fact:** Since vaccines work differently in individuals, it is difficult to predict the level of antibody one may acquire. Non-vaccinated individuals who recover from COVID-19 are twice as more susceptible to reinfections compared to those who recover and receive the vaccine [157].

**Myth:** COVID-19 mRNA vaccines contain microchips

**Fact:** The basic ingredients of mRNA-based vaccines are sucrose, cholesterol, and lipids among others [158].

**Myth:** COVID-19 mRNA vaccines can generate a magnetic field in the body

**Fact:** The vaccine does not contain any metal components and thus cannot create an electromagnetic field anywhere in the body [158].

**Core factors in the sustainability of mRNA vaccines**

**Is efficacy the sole determining factor?**

Based on the COVID-19 mRNA vaccine trials, both the Pfizer/BioNTech and Moderna mRNA vaccines had very similar efficacies of 95% and 94.1%, respectively. Consequently, these candidates were the first mRNA vaccines to achieve FDA ‘emergency use authorisation’ and the European Medicines Agency (EMA) ‘conditional approval’.

In any vaccine trial, efficacy plays a pivotal role in measuring the vaccine’s ability to prevent the indicated disease. Although both the BNT162b2 and mRNA-1273 vaccines had similar efficacies, to better understand the term ‘efficacy’, one needs to look closer at the experimental design of the trials. Variabilities in factors, such as time, population, vaccine regime, course-of-time between vaccinations, and exposure...
can affect the efficacy of any vaccine, inordinately. For example, if a clinical trial is conducted in a region where new pathogenic variants can suppress the vaccine, it is expected to have a lower efficacy rate as the vaccine was originally not generated against that particular variant of the pathogen. However, the vaccine can show better efficacy in a different region where those variants are not dominant in the population during the clinical trial. Therefore, having a lower efficacy does not necessarily indicate that a vaccine is not effective enough against the target disease.

Efficacy is based on the outcome of a controlled clinical trial where the participant pool is aimed to be representative of the population in terms of age, gender, and ethnicity, among other factors. Vaccine effectiveness, on the other hand, is a more real-world measure of a vaccine’s ability to protect against a disease within a wider community where more variabilities exist that cannot be accounted for in a clinical trial.

Therefore, a vaccine’s effectiveness may be different to its efficacy with the ultimate decision of a vaccine’s effectiveness requiring careful consideration of the aforementioned factors that are closely associated with efficacy [159].

Storage and distribution of mRNA vaccines

A significant post-approval hindrance with the COVID-19 mRNA vaccines has been associated with the storage temperature of these vaccines, which is in the range of −20°C to −80°C for long-term storage (three to six months) [160].

Storage at such low temperatures represents a logistical roadblock to the distribution of these vaccines, with the majority of developing and under-developed countries lacking the appropriate infrastructure to support long-term acquisition. Therefore, both the Pfizer/BioNTech and Moderna mRNA vaccines have been beyond the reach of a vast majority of the world population.

However, efforts are underway to counteract this issue regarding storage. For example, CVnCoV, an mRNA vaccine manufactured by CureVac (Tübingen, Germany), used a platform that extends the stability of the mRNA for up to three months at 2–8°C [160]. Other than that, the second generation of CureVac COVID-19 vaccine and ARCoV COVID-19 vaccine (co-developed by the Academy of Military Science, Walvax Biotechnology, and Suzhou Abogen Biosciences), have been reported to be thermostable and can be stored for at least one week at room temperature [161]. Consequently, the approval of these vaccines would significantly change the global distribution of mRNA vaccines.

Recently, BioNTech has further introduced a low-cost mobile production unit that is equipped with 12 containerers called BioNTainer that can produce 50 million doses of mRNA vaccines annually. BioNTech is expected to deliver these modular production units to Africa to combat COVID-19 [162]. Additionally, CureVac is collaborating with Tesla (Texas, USA) to develop ‘RNA printers’ which will be automated RNA vaccine production units [163].

As an alternative to relying on vaccines from multinational pharmaceuticals, WHO has recently established an mRNA vaccine technology hub to manufacture mRNA vaccines in low- and middle-income countries [164]. A prototype COVID-19 vaccine has already been designed independently by the hub and the technology along with the training for bulk production of the vaccine will be transferred to a growing list of countries including Bangladesh, Indonesia, Pakistan, Serbia, Vietnam, Egypt, Kenya, Nigeria, Senegal, Tunisia, Argentina, and Brazil [164,165]. Successful implementation of this strategy will not only help combat COVID-19 but also potentially help in the fight against other diseases.

Since mRNA vaccines are relatively new to commercialisation, better strategies to stabilise these vaccines both at the manufacturers’ and the distributors’ ends are a matter of sustained research efforts and time, as would be expected of any novel and emerging technology.

How long does protective immunity last?

Upon vaccination, the desired antigen is expressed by the targeted cell or taken up by APCs to lymph nodes which initiates the interaction between B cell, APCs, and follicular helper T cells. This interaction supports the formation of the germinal centre. The germinal centre allows the formation and maturation of B cells and immunoglobin class switching to promote the production of high-affinity neutralising antibodies against the targeted antigen and plays a crucial role in prolonged immunity against a disease [166].

However, it is difficult to precisely predict the duration of acquired immunity against COVID-19. The functioning of the immune system inherently varies between individuals even when they are vaccinated with the same vaccine or naturally exposed to the same virus. It has been observed that individuals who recovered from COVID-19 with mild-to-severe symptoms still had antibodies five to seven months post infection [167]. Even though the neutralising antibodies diminish with time, clinical trials of mRNA-1273 vaccine reported high levels of antibody in participants even after six months of their second dose [168]. BNT162b2 vaccine also documented similar antibody levels after six months [169]. It was also shown that even though antibody titres waned with time, B and T memory cells were present even after eight months, indicating the possibility of protection against COVID-19 for a longer period of time [170].

It is also known, based on data from past epidemics and pandemics, that protective immunity may last decades. For example, it was reported that individuals who contracted the 1918 H1N1 influenza virus still retained immunity against the antigen 90 years later [171]. Additionally, individuals who recovered from the more recent 2003 SARS-CoV-1 infection still harboured some CD4+ and CD8+ T cells against the antigen 17 years later [172].

Therefore, even though the immunological analysis of mRNA-based COVID-19 vaccine data is very promising, it is too early to be able to determine the longevity of protective immunity. As such, both time and further comprehensive studies are necessary in this regard.
Prospective mRNA vaccines across therapeutic avenues

With the approval of two mRNA vaccines against COVID-19, widespread acceptance of the technology has begun to come forth. By leveraging rapid development, safety, and high potency over traditional vaccine and therapeutic platforms, mRNA technologies present an immense potential to fight promptly against both infectious and non-infectious diseases.

Cancer

Cancer mRNA vaccines are being extensively studied since they present a promising alternative to conventional chemotherapy. Tumour-associated antigens which are expressed selectively in cancerous cells could be harnessed to design cancer mRNA vaccines [32]. Some key developments in mRNA vaccines for cancers utilise the power of mapping cancer-related mutations in the genome. An example is the ‘mutanome’ approach which is the overall detection and mapping of somatic mutations in tumours of individual patients using next-generation sequencing (NGS) technology [16]. Based on the identified mutations, personalised neo-epitope mRNA cancer vaccines may be designed.Neo-epitopes are small peptides that are derived from tumour-specific somatic mutations that are exposed on cancer cell surfaces and can be recognised by T cells [16,173]. Using this technology, cancer mRNA vaccines may be tailored to individual patients and this technique was successfully employed on melanoma patients [16,174]. Mutanome-based mRNA vaccines for triple-negative breast cancer are currently undergoing clinical trials [175].

Generally, clinical trials merge mRNA vaccines with cytokines or checkpoint modulators to boost immunity. This approach is currently being used in clinical trials to treat malignant and metastatic tumours including melanoma [176–178], glioblastoma [179], non-small cell lung cancer [177,180,181], colorectal cancer [177,180,182], and pancreatic cancer [180,183].

Tumour-associated antigens (TAAs) that are selectively expressed in cancer cells can also be targeted by mRNA vaccines. For example, a mixture of different mRNAs targeting TAAs for melanoma has been used in clinical trials for the treatment of metastatic melanoma. BNT111 (also known as Lipo-MERIT) is a well-known example of an mRNA vaccine in this category. BNT111 targets the TAAs – New York oesophageal squamous cell carcinoma 1 (NY-ESO-1), tyrosinase, melanoma-associated antigen 3 (MAGE-A3), and transmembrane phosphatase with tensin homology (TPTE). Phase one/two trials are currently being carried out to assess the safety, tolerability, and efficacy of this vaccine in patients suffering from advanced melanoma [184,185].

AIDS

Since its discovery in the 1980s, acquired immunodeficiency syndrome (AIDS), a chronic and life-threatening disease caused by human immunodeficiency virus (HIV) infection, has yet to find a truly effective and manageable solution. Defeating HIV is a major focus of research on mRNA vaccines. As such, there are several mRNA vaccines in clinical trials for the treatment of AIDS [32].

One strategy is to use dendritic cell delivery systems which are generally used in cancer treatment. It has been shown that dendritic cell mRNA vaccines activate CD4+ and CD8+ T-cell immune responses although there was little observed benefit during the clinical trials [32]. On the other hand, the combination of mRNA vaccine therapy and immunotherapy may achieve more promising results. This approach is currently being tested in the HIVACAR project. In this phase one/two clinical trial an mRNA vaccine that is tailored to individual patients is combined with a CD4 blocking antibody and a latency-reversing agent (LRA) which reactivates latent HIV-infected cells [186]. If successful, this approach may significantly reduce the costs associated with HIV treatment while improving patient care.

HTI-TriMix is yet another new mRNA vaccine candidate against HIV-1. Activation adjuvant TriMix and mRNA that codes for 16 conservative fragments of HIV structural proteins (Gag, Pol, Vif, and Nef) have been combined to design this vaccine. Upon intranasal injection in mice, potent antigen-specific cytotoxic T-cell responses were elicited [187]. Based on phase one and two clinical trials, this vaccine was found to be safe but the HTI protein expression was affected due to an unexpected start codon found upstream of the sequence that codes for the HTI recombinant antigen [188]. Therefore, further studies are required to overcome this problem. Additionally, there are still difficulties in the treatment of AIDS due to the poor understanding of HIV and the pathophysiology of the disease. However, if proper antigen targeting is employed, mRNA vaccines can play a crucial role in preventing HIV infection.

Influenza

Influenza viruses have a high rate of mutation which has made it difficult to eradicate influenza with traditional vaccines. Since mRNA vaccines can be rapidly and specifically developed against a target variant, they are suitably poised to tackle the high mutation rate of influenza viruses. Phase one clinical trials of two non-replicating mRNA vaccines against influenza viruses (H10N8 and H7N9) have been carried out and both vaccines were found to be safe and effective in eliciting an immune response [189]. Another vaccine platform known as RNAactive, which has self-adjuvant properties, also showed promising results in pre-clinical trials [190,191].

Rabies

Individuals who are more likely to be exposed to the rabies virus are recommended to receive pre-exposure treatment [192]. CV7201 is a prophylactic vaccine for rabies virus glycoprotein (RABV-G) which, in phase one clinical trials, was found to be safe and efficacious. However, there were problems associated with unstable administration-dependent immune responses [193]. More recently, LNP-encased RABV-G mRNA was found to be better at eliciting both humoral and cell-mediated immune responses in preclinical trials with subsequent trials.
being currently pursued [194]. Another candidate, CV202 was also found to be safe and tolerable in patients in addition to meeting the WHO’s requirements in terms of the neutralising antibody titres [195].

Zika virus infection

The lack of a safe and effective vaccine is hampering efforts to tackle Zika virus (ZIKV) infections. However, one potential vaccine has been suggested to be a nucleoside-modified mRNA that encodes viral premembrane and envelope glycoproteins. In the presence of the modified nucleoside, N\(^{3}\)-methylpseduouridine, an mRNA encoding the prM-E region of the ZIKV H/PF/2013 was designed. Upon a single intradermal injection of the LNP-encased mRNA, significant neutralising antibody titres, as well as ZIKV-specific cellular responses, were mounted in immunocompetent mice and rhesus macaques, thereby providing full protection against the viral challenge [196].

Additionally, the vaccines mRNA-1325 and mRNA-1893 have been shown to protect against ZIKV-induced congenital disease in both immunocompetent and immunocompromised mice during pregnancy [197,198]. For both these candidates, phase one clinical studies have recently been completed, thereby paving the way for potential vaccinations in humans [199,200].

Respiratory syncytial virus infection

Respiratory syncytial virus (RSV) is a leading cause of respiratory tract infection among infants and elderly individuals and it is responsible for approximately 74,000 deaths per year [201,202]. In 1968, a formalin-inactivated RSV vaccine candidate generated vaccine-associated enhanced diseases (VAED) which resulted in severe pneumonia or bronchiolitis in 80% of the vaccinated children with two fatalities [203]. To this day no working vaccine against RSV has been developed.

Current vaccine efforts utilise the viral surface protein F which aids the fusion of the virus with the host cell membrane. Even though a few such vaccine candidates failed due to insufficient neutralising antibodies [204], a novel insight into the protein F structure has revealed that the use of perfusion conformation enhances neutralising antibodies [205-208]. In preclinical studies, mRNA vaccines encoding either the stabilised perfusion conformation or the native protein F were delivered without any VAED indication in mice and rats [83,209].

Moderna is currently developing three RSV vaccines which include mRNA-1172 and mRNA-1777 for adults and mRNA-1345 for children. Phase one clinical trial of mRNA-1777 exhibited humoral immunity with neutralising antibodies and CD4+ T cell-mediated responses against protein F without any severe side effects [210]. In mRNA-1345, further codon optimisation was used to extend translation efficiency and immunogenicity to levels that were similar to that of mRNA-1777. Interim data of phase one clinical trial shows that a 100 μg dose of mRNA-1345 vaccine, post one month, provides eightfold higher neutralising antibodies than mRNA-1777 [34]. These data highlight the potential of mRNA vaccines towards helping eradicate RSV infections where other vaccine regimes have failed to achieve significant success.

Malaria

Even though mRNA vaccines are primarily targeted against a wide range of viruses, efforts are also underway in exploiting this platform to fight other pathogens. *Plasmodium*, a genus of unicellular eukaryotic parasites, is the primary cause of malaria which leads to 400,000 deaths globally [211]. The lack of a target surface protein as well as the complicated life cycle of *Plasmodium* has so far hindered the development of an effective vaccine against malaria. However, recent studies on understanding immune responses against *Plasmodium* have revealed new antigen targets.

Macrophage migrating inhibitory factor (PMIF), a cytokine secreted by *Plasmodium*, has been identified to avert T cells from generating long-term memory responses [212]. This led to the design of a self-amplifying mRNA vaccine encoding PMIF which is complexed with squalene-based cationic nanoemulsion. Upon successful administration of two 15 μg-doses of PMIF mRNA, anti-*Plasmodium* antibodies were generated with memory T cell responses [212]. Subsequently, it was identified that *Plasmodium falciparum* glutamic acid-rich protein (PfGARP), which binds to the surface of infected erythrocytes, causes programmed cell death. This further led to the formulation of another nucleoside-modified mRNA vaccine encoding PfGARP. Upon LNP-mediated delivery of three 50 μg doses of the vaccine, parasitaemia was reduced in Aotus monkeys [213].

Lyme disease

Lyme disease is a growing concern in North America and Europe caused by the black-legged tick, *Ixodes scapularis* which acts as a medium for the transmission of a spectrum of pathogens including *Borrelia burgdorferi*, the causative agent of Lyme disease. In some animals, resistance to tick infections can be achieved by repeated exposure to ticks but this has not been demonstrated for humans, thereby necessitating a working vaccine [214].

Recently, a nucleoside-modified mRNA vaccine encoding 19 salivary proteins of *I. scapularis* (19ISP) was designed and injected into guinea pigs where it led to potent antibody responses. Subsequent exposure to uninfected *I. scapularis* nymphs in the vaccinated animals led to poor feeding and detachment of the ticks with 80% detachment in vaccinated animals compared to 20% in unvaccinated animals [215].

Multiple sclerosis

Multiple sclerosis (MS) is one of the leading causes of neurological disorders in young adults, likely disabling the brain and spinal cord with 2.2 million people affected globally [216]. Recently, a nucleoside-modified mRNA vaccine was tested in a mouse model of MS. In this disease model, the myelin oligodendrocyte glycoprotein epitope, MOG35-55, induces MS pathology. Therefore, LNP-encased N\(^{3}\)-methylpseduouridine-containingmRNA encoding MOG35-55 was successfully
administered to mice. Consequently, there was a suppression of effector T cell populations and activation of regulatory T cell populations which protected the mice from developing MS [217].

**Transthyretin amyloidosis**

Transthyretin amyloidosis, also known as ATTR amyloidosis, is a potentially fatal illness marked by the accumulation of misfolded transthyretin (TTR) protein fibrils in internal organs [218]. NTLA-2001 is an in vivo gene-editing therapeutic approach that is intended to cure ATTR amyloidosis by lowering serum TTR levels. It is based on the clustered regularly interspaced short palindromic repeats and Cas9 endonuclease (CRISPR-Cas9) system and consists of LNP-encased Cas9 mRNA and a single guide RNA targeting TTR. TTR knockdown was demonstrated to be persistent after a single dosage in preclinical trials with minimal adverse events, thereby highlighting the efficacy of this strategy in ATTR therapy [219].

**Allergies**

mRNA vaccines pave the way for a safer preventative immunisation against allergic conditions. mRNA vaccines encoding the allergen ensure higher purity of the immunising antigen compared to more traditionally used allergen extracts which may initiate further allergic responses [220].

mRNA vaccines are efficacious as preventative vaccines against type I allergy in mice due to activation of a Th1 cell response [221]. The robustness of this Th1 response has also been demonstrated in mice that were injected with mRNAs encoding a wide variety of allergens. Following immunisation with a particular mRNA, the mice were challenged with the respective allergen. Ultimately, it was observed that inflammatory signatures such as eosinophil count, IL-4 and IL-5 levels were reduced whereas anti-inflammatory phenotypes such as induction of IFN-γ producing cells were enhanced [222].

It has further been demonstrated that mRNA vaccines may be able to elicit long-term memory responses in immunised mice with subsequent re-exposure to the allergen inducing potent anti-inflammatory responses [223]. This also highlights the potential of using mRNA vaccines to target allergies without the requirement of booster vaccinations.

Overall, mRNA vaccines possess the ability to bring forth the next generation of futuristic medicine with genome editing and gene therapy for the aforementioned diseases among others. As such, significant financial investments have been made in the field of mRNA vaccine technology (Box 3). As of today, there are several mRNA vaccines and therapeutic candidates for different diseases and medical conditions in clinical trials, as indicated in Table 2. Despite this, it is unlikely that any changes would occur in the already approved and cost-effective vaccine platforms for polio, hepatitis B, chickenpox, and diphtheria, among others.

**Box 3**: Outlook on the economic impact of mRNA vaccines

Borne out of the COVID-19 pandemic, appreciation of mRNA vaccines has initiated substantial investments in the technology. There has been a sharp growth of combined market capitalisation for five publicly listed mRNA companies in the USA from USD 15 billion in 2019 to USD 300 billion in 2021, indicating the economic impact of mRNA vaccines [224].

This boost is further backed by the government and non-government funding agencies in North America and Europe which constituted cumulative global funding in 2020 of over USD 9 billion for mRNA therapeutics [225]. In 2021, two new mRNA start-ups, Laronde (Cambridge, USA) and Orna Therapeutics (Cambridge, USA) secured USD 440 million and USD 80 million, respectively [226]. Early investments of this scale reflect the triumph of mRNA vaccines against COVID-19 with the market value solely based on COVID-19 vaccines estimated at USD 50 billion in 2021 [224].

Multinational pharmaceutical companies are also investing in the research and development of mRNA vaccines. Sanofi S.A. (Paris, France) recently acquired a clinical-stage mRNA therapeutic company, Translate Bio (Massachusetts, USA) with USD 3.2 billion [227]. Additionally, Sanofi S.A. plans to annually invest USD 400 million in its one-of-a-kind ‘mRNA Center of Excellence’ [228]. Furthermore, Novartis (Basel, Switzerland) has signed a contract with BioNTech for the bulk production of BioNTech’s COVID-19 vaccine in 2022 [229]. GlaxoSmithKline (Brentford, UK) has also initiated a €150 million collaboration with CureVac to develop the second generation of COVID-19 vaccines to address the emerging SARS-CoV-2 variants [230].

All of these collaborations indicate the interest of global healthcare companies in mRNA-based vaccines and therapeutics which may significantly play a role in expanding its market. However, there is a possibility of a decline in the mRNA vaccine market from 2023 to 2025 due to decreased demand for COVID-19 vaccines as a result of the waning pandemic. Despite this, the affirming nature of COVID-19 vaccines could facilitate efforts to address the lag that currently exists in clinical trials for other prospective mRNA vaccines. As such, it may be expected that from 2028 onwards new mRNA vaccine products targeting a myriad of diseases will come forth [224].

By 2035, the projected market value is set to reach USD 23 billion with prophylactic vaccines dominating by generating 50% of the revenue while therapeutic vaccines and other therapeutics may account for 30% and 20% of the profits [224].

**Conclusion**

From their early indication as a therapeutic tool in the 1990s to exhibiting their flair as a pioneering vaccination programme in 2020 amidst one of the greatest pandemics in recent times, mRNA vaccines have traversed difficult roadblocks to deliver their promise. In line with the success of COVID-19 mRNA vaccines, initiatives are being undertaken by the government, corporations and academia to further utilise this exemplary vaccine regime for other diseases. As such, the future of vaccines, gene therapy, and genome editing are likely to be hinging on mRNA technologies. With the foundation set by the approved COVID-19 mRNA vaccines, faster approval of future vaccines will lead to a further demystification of the technology. However, the bench-to-bedside translation of mRNA vaccines will necessitate adequate funding, international collaborations between policymakers, industry, and academia as well as a consensus within the public health community. Although some of these requirements often tend to be unmet in a pandemic-free world, their overall importance has received prominence during the challenging times of COVID-19. With a growing number of mRNA vaccine candidates entering clinical trials and production pipelines, the cloud of scepticism surrounding this technology
| Table 2. mRNA-based vaccines and therapeutics in clinical trials. |
|-------------------------------------------------------------|
| **Infectious disease vaccines**                              |
| Antigen encoded by mRNA                                      | Route of administration | Target condition | Phase | Status               | Clinical trial number |
| mRNA-1273 Full-length, prefusion-stabilised spike (S) protein of SARS-CoV-2 | Intramuscular           | COVID-19          | Phase 4 | Recruiting            | NCT04780659          |
| Full-length, membrane-anchored spike (S) protein of SARS-CoV-2 | Intramuscular           | COVID-19          | Phase 4 | Recruiting            | NCT04760132          |
| ChulaCov19 Undisclosed                                      | Intramuscular           | COVID-19          | Phase 1 | Recruiting            | NCT04674189          |
| PTX-COVID19-B Full-length, membrane-anchored spike (S) protein of SARS-CoV-2 | Intramuscular           | COVID-19          | Phase 2 | Recruiting            | NCT04668327          |
| CoV2 SAM Spike (S) protein of SARS-CoV-2                   | Intramuscular           | COVID-19          | Phase 1 | Recruiting            | NCT04758962          |
| mRNA-1273.351 Full-length, prefusion-stabilised spike (S) protein of B.1.351 variant | Intramuscular           | COVID-19          | Phase 1 | Recruiting            | NCT04798027          |
| MRT5500 Full-length, prefusion-stabilised spike (S) protein of SARS-CoV-2 | Intramuscular           | COVID-19          | Phase 1 | Recruiting            | NCT03829384          |
| CV7202 Rabies virus glycoprotein, RABV-G                    | Intramuscular           | Rabies            | Phase 1 | Recruiting            | NCT03164772          |
| mRNA-1647 Subunits of the CMV pentamer complex and the glycoprotein B (gB) protein | Intramuscular           | Chikungunya       | Phase 1 | Recruiting            | NCT03829384          |
| mRNA-1944 A fully human IgG antibody originally isolated from B cells of a patient with a prior history of potent immunity against Chikungunya infection | Intravenous             |                   | Phase 1 | Recruiting            | NCT03829384          |
| **Cancer vaccines**                                         |
| Antigen encoded by mRNA                                      | Route of administration | Target condition | Phase | Status               | Clinical trial number |
| CV8102 A noncoding single-stranded RNA complexed with a cationic peptide that functions as a strong immunomodulator based on TLR7/8 and RIG-1 activation | Intratumoral           | Cutaneous melanoma, adenoscydotic carcinoma, squamous cell cancer of skin, head, and neck | Phase 1 | Recruiting            | NCT03291002          |
| mRNA-4157 Personalised cancer vaccine (PCV) with a combination of validated defined neoantigens, predicted neoepitopes, and mutations in driver genes | Intramuscular           | Solid tumours     | Phase 1 | Recruiting            | NCT03313778          |
| mRNA-5671/ V941 Four common mutations of an oncogene known as KRAS | Intramuscular           | Non-small cell lung cancer, pancreatic neoplasms, and colorectal neoplasms | Phase 1 | Recruiting            | NCT03948763          |
| mRNA-5671/ V941 Four common mutations of an oncogene known as KRAS | Intramuscular           | Non-small cell lung cancer, pancreatic neoplasms, and colorectal neoplasms | Phase 1 | Recruiting            | NCT03948763          |
| **Curative and protein supplementation therapeutics**       |
| Antigen encoded by mRNA                                      | Route of administration | Target condition | Phase | Status               | Clinical trial number |
| AZD8601 Vascular endothelial growth factor A (VEGF-A)        | Epicardial              | Myocardial ischemia | Phase 2 | Recruiting            | NCT0370887          |
is starting to clear. Like every monumental therapeutic platform, mRNA vaccines have their limitations. However, massive strides have been made in adapting them for better efficacy, safety, delivery, and tolerance with efforts on this front remaining a continued research priority. With well-equipped manufacturing capabilities for mRNA-based vaccine production worldwide, thorough guidelines can be expected for the production and evaluation of this new age of therapeutics in the years to come.

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N.Z.R., S.B., and N.T. are shared first authors and have contributed equally to the conceptualisation and writing of the article. N.T. has also been involved in the drafting and editing of the article. Additionally, S.B. has designed Figure 4 with BioRender. L.M.O. is the second author and has illustrated Figures 1, 2, and 3 and written the section ‘The concept of mRNA vaccines’. M.M.M. is the third author and has written the ‘Introduction’ and assisted N.Z.R., S.B., and N.T. in the design of tables.

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