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A Review On mRNA Vaccines And Their Pharmaceutical Impact

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Abstract:- The introduction of mRNA vaccines are a powerful alternative to standard practices accepted vaccines for many reasons including: potent, safe and efficacious induced immunity, rapid clinical development, and rapid and economical manufacture. These vaccines are no longer a curiosity; rather they are earning their place as the pandemic vaccine platform. Advances in delivering systems (i.e. LNP and other nanotechnology) for developing mRNA vacancies are exciting and important. This review is an examinable history of every aspect of the mRNA vaccine for infectious diseases. The article focus on mRNA structure, pharmacological use and function of immunity induction, lipid nanoparticles (LNP), from upstream, through downstream, and formulation of mRNA vaccines for manufacture. The article is specific to mRNA vaccines in clinical trials. The article looks at the future and potential of mRNA vaccines freeze-drying, eyewear delivery systems, and LNP characteristics which target antigen presenting cells and dendric cells.

Keywords:- mRNA structure; mRNA immunogenicity; mRNA vaccines clinical trials; lipid nanoparticles (LNPs); cationic lipids; ionizable lipids; PEGylated lipids; lyophilization of mRNA vaccines; adjuvants; antigen presentations

1. Introduction

Vaccines are a major milestone for humanity in terms of limiting the spread of communicable disease. Vaccines also have a tremendous impact on the economic viability of the health care system because they reduce lowering treatment costs for communicable disease. Vaccination further researches on impact and the risk of outbreaks [1,2], overall survey of vaccination in a more broad perspective as used in public health and safety is not all aspects of it economic impact immensely surfaced with the emergence with COVID-19 [2]. Vaccination campaigns have met success when there is eradication of life-threatening illnesses including smallpox, polio, and an effort with COVID-19. The WHO estimates that vaccines prevent between 2–3 million deaths per year from pertussis, tetanus, influenza, and measles [3]. Vaccines have taken a path from the use use of inactivated and/or attenuated pathogens to subunit vaccines which consists only components of a target pathogen for eliciting an immune response. Important milestones in vaccine research, but not limited to, are the development of recombinant viral-vector vaccines, virus-like particle vaccines, conjugated polysaccharide- or protein-based vaccines, and

toxoid vaccines. Notably, the most important and milestone was the development of mRNA-based vaccines because of its swift development and licensure for the COVID-19 pandemic and its mRNA technology producing the requisite vaccine antigen intracellularly. We currently live in the world of mRNA vaccinations, because basic research had already been performed over three decades ago [4,5].

Although effective efforts were made in the 1990's to develop an effective in vitro transcribed (IVT) mRNA vaccine in animals' epitope presentation [6,7], mRNA vaccines and therapeutics were developed nor validated until later in the 1900's. In the last decade, major technological advances and investigations in improving overall mRNA quality have conducted by

(i) enhancing its stability through capping, tailing, point mutations, and purification methods,

(ii) enhancing mRNA delivery by using lipid nanoparticles

(iii) lessening its immunogenicity through the consideration of modified nucleotides, has led to its application as a vaccine. mRNA vaccines have many notable benefits when compared to traditional vaccines which include live and attenuated pathogens, subunit-based, and DNA-based vaccines. These benefits include

(i) safety, because mRNA will not integrate with host DNA and is non-infectious;

(ii) efficacy due to modifications in the structure of mRNA can customize the stability and efficacy of the vaccine, with less immunogenicity; and

(iii) greater production and scaleup efficiencies because mRNA vaccines are produced in a cell-free environment, allowing for rapid, scalable, and cost-effective production. A 5 L bioreactor can also produce 1 million doses of an mRNA vaccine in a single reaction (Himanshu et al., 2021). Furthermore, mRNA vaccines have the capability of coding for many more antigens, subsequently producing a stronger immune response to some resilient pathogens[9].The efficacy of this vaccine technology was realized when mRNA vaccines were developed and approved by Pfizer–BioNTech for the COVID-19 pandemic. These vaccines were developed in a record-short time of under a year, after the world was gripped with SARS-CoV-2 virus infection, causing hospitalization and death. The unprecedented development of Spikevax® (Moderna) and Comirnaty® (Pfizer–BioNTech) and its mass vaccination to millions of people helped to control the COVID-19 outbreak.

The manufacturers of these vaccines have demonstrated an ability to develop, approve and manufacture mRNA vaccines; therefore, the use of this platform as a vaccination technology has been established as safe and effective. Furthermore, interest in the scientific community regarding mRNA as a prophylactic vaccine technology has increased markedly.elf-amplifying mRNA vaccines; safety; efficacy; acceptability. In this review, we provided a basic summary of mRNA vaccines, including its mRNA structure and pharmacological engagement, mRNA structural modifications, and described how mRNA vaccines stimulate and elicit a desired immune response in the host. The review also covered the role of lipid systems including lipid nanoparticles as mRNA delivery systems. This review outlined the detailed structural make-up and mechanism of action of lipid nanoparticles. New developments in second-generation mRNA vaccines as well as information on going clinical trials were also included.

Historical Background of Vaccines:-

The origins of vaccines date back to the 1790s with the development of smallpox vaccination. Traditional immunization strategies relied heavily on whole-pathogen approaches such as live attenuated or inactivated vaccines.

1.1 Types of vaccines:-

There are several types of vaccines based on how they are made and how they stimulate immunity:

1. Live Attenuated Vaccines
2. Inactivated Vaccines
3. Subunit, Recombinant, Polysaccharide, and Conjugate Vaccines
4. Toxoid Vaccines
5. mRNA and DNA Vaccines

1. **Live Attenuated Vaccines:-** Contain weakened forms of the virus/bacteria. Provide strong, long- lasting immunity. (e.g., MMR, BCG)

2. **Inactivated Vaccines:–** Made from killed pathogens. Safer but may require booster doses. (e.g., polio, hepatitis A)

3. **Subunit, Recombinant polysaccharide and conjugate vaccines:-** Use specific parts of the pathogen to trigger immunity. Suitable for those with weakened immunity. (e.g., hepatitis B, HPV)

4. **Toxoid Vaccines:-**Use inactivated toxins (toxoids) produced by bacteria. Prevent diseases caused by toxins. (e.g., diphtheria, tetanus)

5. **mRNA and DNA Vaccines:-** Use genetic material to instruct cells to make a viral protein that triggers immunity. Rapidly developed and used in COVID-19. (e.g., Pfizer-BioNTech, Moderna)

1.2 Traditional Vaccine Technologies:-

Traditional vaccines have relied on culturing pathogens and rendering them safe for administration. Examples include the polio, hepatitis A, and BCG vaccines. These methods require extensive safety and stability studies.

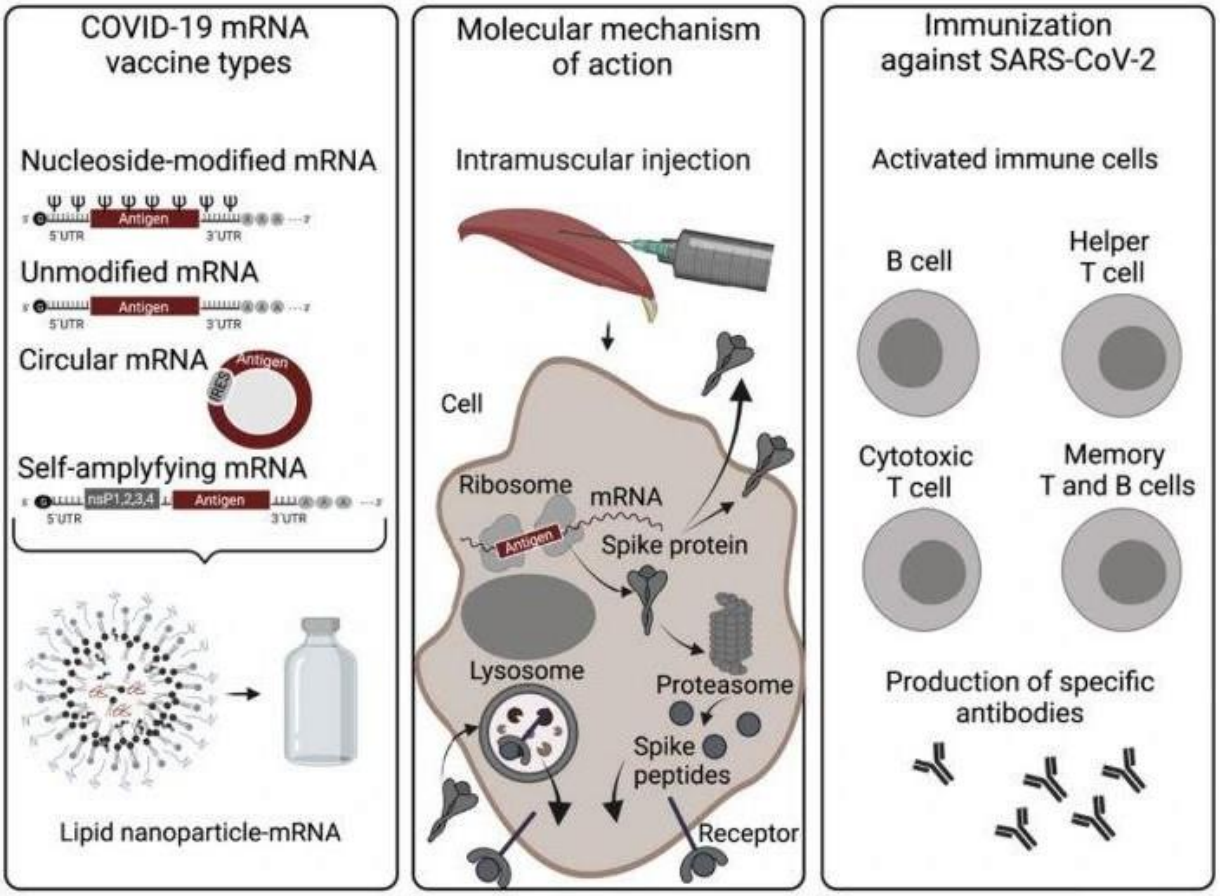
1.3 The Rise of mRNA Vaccines:-

mRNA vaccines represent a shift from classical vaccine platforms. They work by instructing host cells to produce a viral protein that triggers an immune response. Pfizer-BioNTech and Moderna's COVID-19 vaccines are prominent examples.

1.4 Mechanism of mRNA Vaccines:-

mRNA is delivered via lipid nanoparticles into the host cells. These cells then translate the mRNA into viral antigens, which are recognized by the immune system, thereby building immunity without using live virus.

Fig.1 Types of COVID-19 mRNA vaccines, their molecular mechanism of action, and how they provide immunization against SARS-CoV-2



1.5 Advantages and Limitations of mRNA Vaccines:- Advantages:-

- Rapid development and scalability

- Non-infectious and synthetic

- Strong immune response

Limitations:-

- Cold chain requirements

- Limited long-term data

- High cost and storage issuses

1.6 Cold Chain Management and Distribution:-

Cold chain refers to maintaining appropriate temperature conditions for vaccine storage and transportation. mRNA vaccines, especially during COVID-19, posed major logistical challenges requiring ultra-low temperature storage.

1.7 Comparative Analysis of Traditional vs. mRNA Vaccines:-

Traditional vaccines are time-tested and easier to store, but take longer to develop. mRNA vaccines allow rapid response during pandemics but are technically demanding.

1.8 Regulatory Challenges in Vaccine Development:-

All vaccines must undergo rigorous preclinical and clinical trials. Regulatory agencies such as WHO, USFDA, EMA, and CDSCO have stringent protocols for approval, especially for new platforms like mRNA.

1.9 Impact of COVID-19 on Vaccine Innovation:-

The pandemic accelerated global collaboration, funding, and regulatory approvals. mRNA technologies gained prominence and could potentially be adapted for other diseases like influenza, HIV, and cancer.

1.10 Future Prospects in Vaccinology:-

Future vaccine strategies may include needle-free delivery, thermostable vaccines, AI-guided design, and universal vaccines for multiple strains or diseases.torage issues

1.11 Pharmaceutical Impact of mRNA Vaccines

1. Drug Development and Innovation

mRNA vaccines introduced a new drug platform that uses genetic code instead of traditional proteins or weakened pathogens.They speed up research and development; mRNA vaccines can be designed in weeks, while conventional vaccines can take months or years.They established the potential for personalized medicine, such as cancer vaccines tailored to individual patients.

2. Manufacturing and Formulation

The focus has shifted to biotechnological manufacturing, using in-vitro transcription instead of cell culture. These vaccines require lipid nanoparticles for drug delivery, driving innovation in nanomedicine. They also encouraged the development of cold chain logistics, such as the need for -70°C storage for the Pfizer/BioNTech vaccine.

3. Regulatory and Quality Assurance

mRNA vaccines paved the way for faster approvals in emergency situations. There is a growing demand for new guidelines to assess the stability, safety, and effectiveness of nucleic acid-based drugs.These developments have led to updates in monitoring systems to track long-term effects.

4. Market and Economic Impact

The vaccines created multibillion-dollar markets for companies like Pfizer, Moderna, and BioNTech. They attracted significant investment in mRNA technologies, not only for vaccines but also for therapeutic proteins, rare diseases, and cancer treatments.This shift has increased collaboration between pharmaceutical companies and biotech start ups.

5. Clinical and Therapeutic Applications

The scope of mRNA vaccines has expanded beyond infectious diseases to include:

- Oncology (cancer immunotherapy)
- Rare genetic disorders
- Autoimmune diseases

These vaccines might also replace monoclonal antibody therapy in some cases.

6. Challenges and Limitations

Stability remains a problem; mRNA is fragile and requires advanced formulations. Storage and distribution are complicated due to the need for ultra-low temperatures.Production costs are higher than for some traditional vaccines. Public acceptance poses challenges, with concerns about safety, side effects, and long-term immunity.

mRNA vaccines represent a major change in how drugs are designed, developed, and delivered. They transformed the vaccine industry and opened new possibilities for gene-based therapies, marking a significant innovation in modern pharmaceuticals.

2. The mechanism of immunization with mRNA vaccines and selection of the SARS-CoV-2 antigen:-

Immunization with mRNA vaccines brings a transcript encoding an antigen in lipid nanoparticles (LNPs) for delivery to antigen-presenting cells (APCs).

LNP-mRNA is taken up by endocytosis and released by endosomal escape into the cytoplasm, where the antigen of interest is generated and presented as a membrane antigen by the transfected muscle and APCs, activating B cells, CD4+ helper T cells, and CD8+ cytotoxic T cell responses in the process.

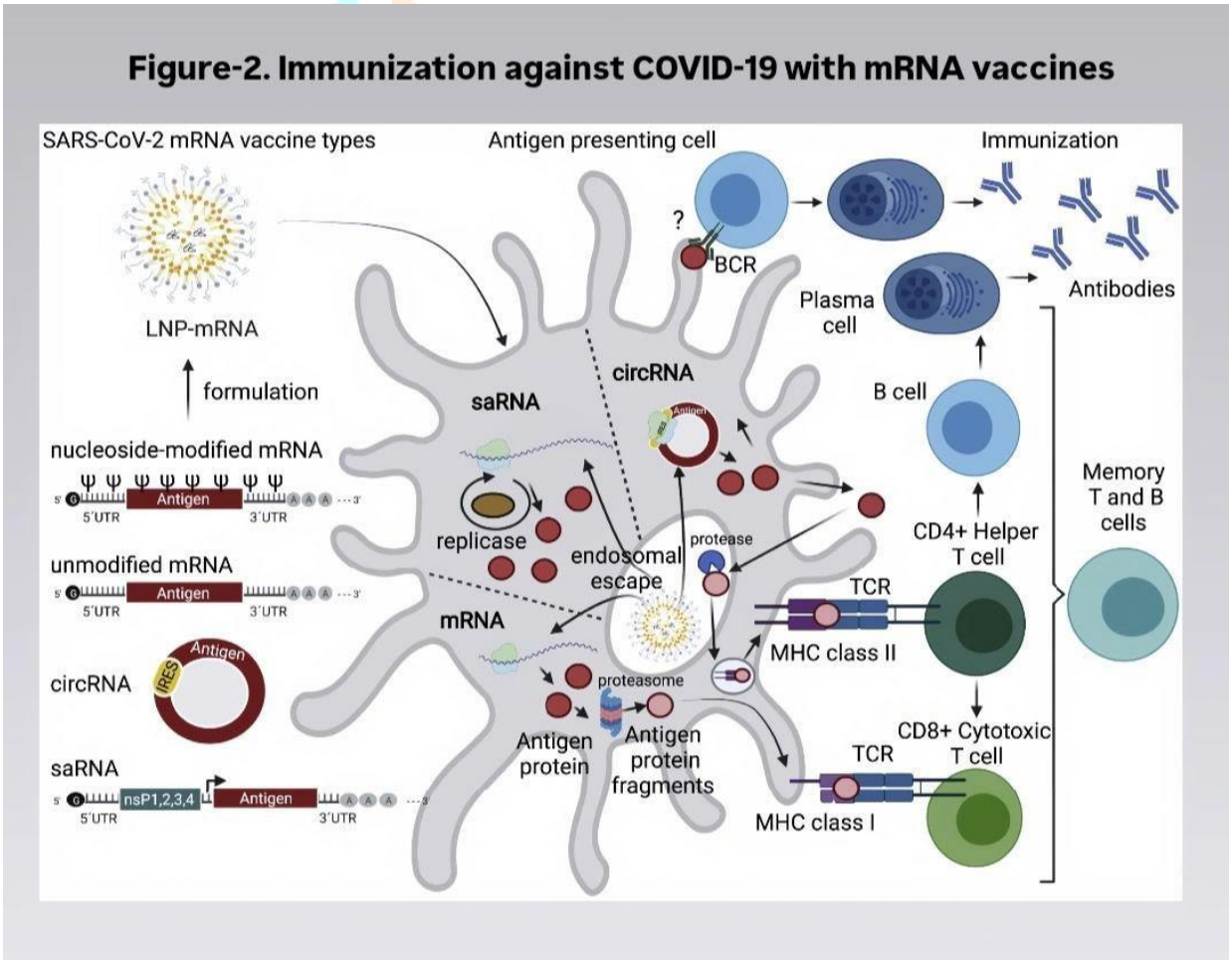
The germinal center B cell response and its regulation by CD4+ T follicular helper (Tfh) cells are vitally important for the development of high-affinity neutralization antibody titers and long-lasting B cell responses[53,54].

Tfh cells bind antigens on the APC surface, which facilitates B cell activation that results in the production of high affinity, virus neutralizing antibodies[6,55,56, 57].

Most recently, the LNP portion of LNP-mRNA vaccines was found to have adjuvant activity depending on its ionizable lipid component and induction of IL-6 cytokines[33]. This LNP-mediated adjuvant activity elicits strong Tfh cell responses and humoral immunity, enhancing mRNA-based vaccine efficacy. Tfh cells also help activate CD8+ cytotoxic T cells that can specifically recognize and eliminate virus-infected cells.

After vaccination with SARS-CoV-2 LNP-mRNA in humans, a durable antigen-specific germinal center B cell response and plasmablast response in blood and draining lymph nodes is elicited, resulting in durable and robust humoral immunity[53,58].

Figure-2. Immunization against COVID-19 with mRNA vaccines



The Pharmacology of mRNA Vaccines

2.1 mRNA Structure

An mRNA molecule allows for efficient translation of the DNA genetic code to cell ribosomes in the cytoplasm to produce proteins. There are two primary mRNA types under evaluation as candidate antigens for vaccine use, non-replicating mRNA and self-amplifying RNA (see Chapter 5). The non-replicating mRNA vaccines encodes the antigen of interest for the immunogenic response, and the 5' and 3' untranslated regions (UTRs) and open reading frame (ORF), also known as the coding region, and poly(A) tail. The self-amplifying mRNA has all of these components along with a RNA coding region in the ORF that encodes for viral replication machinery to allow for constant intracellular RNA amplification and the production of that antigen. In vitro transcription (IVT) is an enzyme catalyzed reaction to prepare a mRNA sequence us.

2.2. 5' Cap

The 5' end of mRNA, known as the 5' cap, has a 7-methylguanosine (m7G) structure followed by a triphosphate group that represents the first nucleotide (m7GpppN). The 5' cap protects RNA from being broken down by exonucleases. It also helps with pre-mRNA splicing and starts the translation of mRNA, as well as its movement from the nucleus to the cytoplasm [10]. The 5' cap is crucial for the innate immune system, as it helps differentiate between non-self or foreign mRNA and the hosts own mRNA [11]. Additionally, mRNA can be modified to improve its effectiveness and stability through various post- transcriptional changes.

Some of these include 20-O-methylation at position 20 of the ribose ring at the first nucleotide (Cap 1, m7GpppN1m) and the second nucleotide (Cap 2, m7GpppN1mN2m). These changes in the 5' cap structure not only improve the translation efficiency of mRNA but also prevent the activation of endosomal and cytosolic receptors, including RIG-I and MDA5, which serve as defenses against viral mRNA [11,12]. Therefore, the 20-O-methylation of the 5' cap structure is a beneficial feature for increasing protein production from the mRNA after transcription and for blocking unwanted immune responses from the host immune system to the antigenic IVT mRNA.

Achieving this 5' cap requires adding S-adenosyl methionine and the Cap 0 structure to the IVT mRNA reaction. This process produces IVT mRNA with the Cap 1 structure and S-adenosyl-L-homocysteine. Cap 1 refers to m7GpppNm, where Nm stands for any nucleotide with a 20-O-methylation. Trinucleotide cap analogs can also create Cap 1 analogs in a co-transcriptional reaction. Ishikawa et al. used m7GpppAG analogs to cap IVT mRNA. These analogs allowed the mRNA to have the m7G moiety at the 5' end without reverse-capped 50 END mRNA products. Further modifications using nucleotides such as A, Am, m6A, or m6Am led to enhanced IVT mRNA specificity. Specifically, the m7Gpppm6AmG cap resulted in the highest luciferase expression in in vitro transfection experiments in cells [13].

Sikorski et al. compared the effects of changing the first transcribed nucleotide, such as A, m6A, G, C, and U, with or without the 20-O-methylation in the mRNA IVT reaction. They found that mRNA delivered by lipofectamine and carrying A, Am, or m6Am as the first nucleotide produced higher luciferase expression, while IVT mRNA with G or Gm resulted in lower luciferase expression. Notably, the mRNA translation in a dendritic cell (DC) line, JAWSII, showed an 8-fold difference between m6A and m6Am 5' caps. These results highlight the importance of the 5' capping structure for effectively targeting DCs and generating the desired immune response [14]

2.3. 5' and 3' UTRs

Although UTRs do not translate into the desired antigen or protein, they play a role in regulating mRNA expression. These regions are found between the ORF and the 5' and 3' ends, both upstream and downstream of the mRNA. UTRs contain regulatory sequences that are linked to mRNA stability and the efficient, correct translation of mRNA. They also aid in ribosome recognition of mRNA and support post- transcriptional modification of the mRNA [15]. Including cis-regulatory sequences in the UTRs can improve mRNA translation and its half-life. Furthermore, naturally occurring sequences like those from alpha- and beta-globins have been commonly used to design mRNA constructs for vaccines [16, 17]. Zeng et al. created de novo 50 UTR sequences based on the guanine-cytosine (GC) content and its length for developing mRNA vaccines [18].

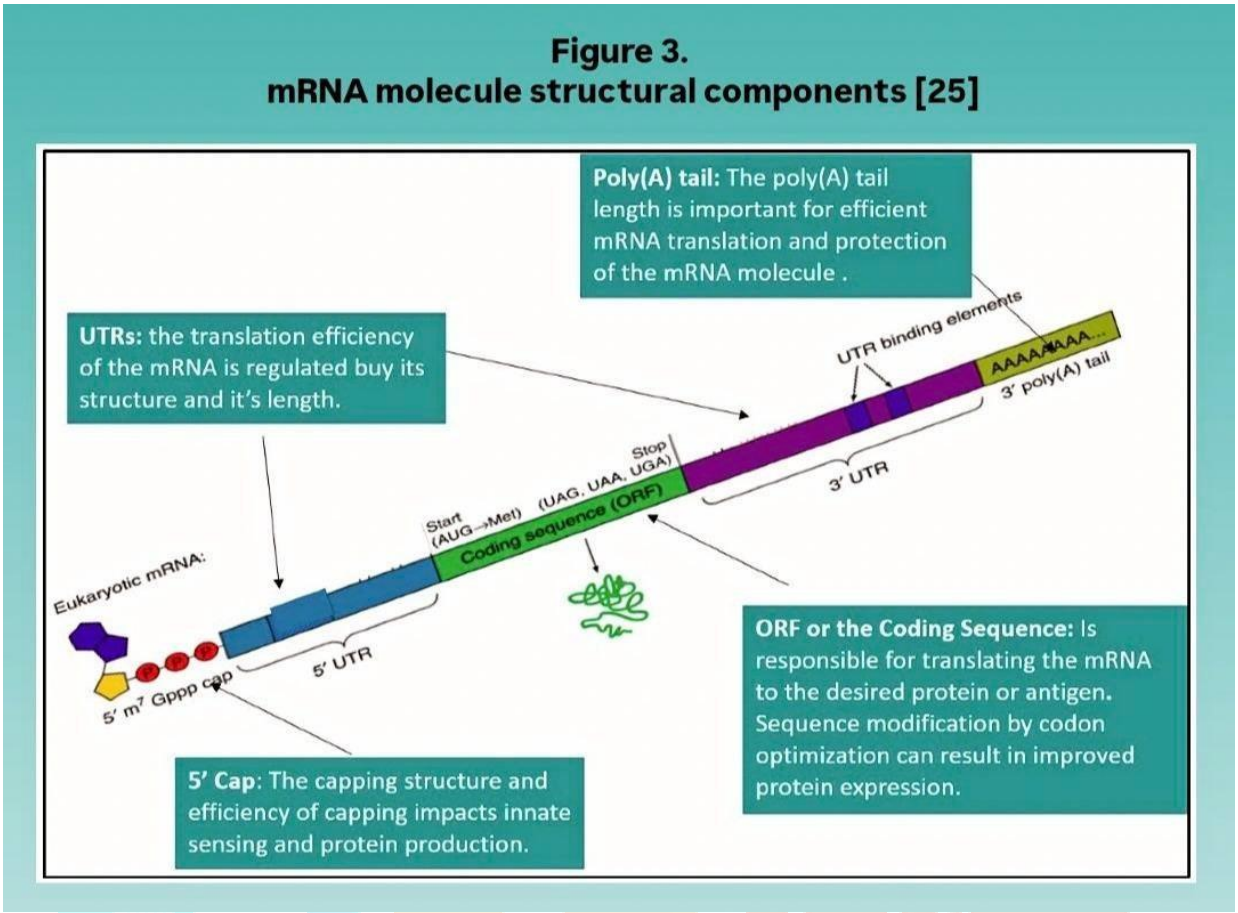
2.4. Poly(A) Tail

The IVT mRNA has a polyadenylated section at its 30 end known as the poly(A) tail. This tail is crucial for determining the lifespan of the mRNA. The poly(A) tails of naturally occurring mRNA molecules in mammalian cells are about 250 nucleotides (nt) long and shorten gradually during the mRNA's life in the cytosol [19]. Since the tail size influences the degradation of mRNA, adding around 100 nt to the poly(A) tail can lead to mRNA with a longer lifespan [20]

2.5. Modified Nucleotides

Natural mRNA and other RNA molecules contain ATP, CTP, GTP, and UTP as the four basic nucleotides. After the post-transcriptional modification of mRNA molecules, some of the nucleotides get changed, like pseudouridine and 5-methylcytidine. These modified nucleotides can be used in the IVT transcription of mRNA. Although non-modified mRNA has its own advantages, modified nucleotides are helpful because they can prevent the innate immune system from recognizing IVT mRNA. This helps avoid unwanted immune responses and improves the translation efficiency of the mRNA into the desired antigen. Andries et al. showed that mRNAs containing the N(1)-methyl-pseudouridine (m1Ψ) modification performed better than the pseudouridine (Ψ)-modified mRNA platform. They provided approximately 44-fold and 13-fold higher reporter gene expression when transfected into cell lines or mice, respectively. The authors also found that m(5C/) m1Ψ-modified mRNA reduced intracellular innate immunogenicity during in vitro transfection. This modification leads to controlled activation of the toll-like receptor 3 (TLR3) and starts downstream innate immune signaling, which is a desired feature of an mRNA vaccine. Figure 1 describes the structural components of an mRNA molecule.

Figure 3.
mRNA molecule structural components [25]



2.6. Innate and Adaptive Immune Stimulation by mRNA Vaccines

A vaccine that includes a pathogen-specific immunogen encoding the viral protein and an adjuvant can support adaptive immune responses. The adjuvant aims to stimulate the innate immune response and signal T cell activation. An ideal adjuvant should trigger the innate immune response without causing systemic inflammation, which can lead to severe side effects. In mRNA vaccines, the mRNA molecule acts as both the immunogen and the adjuvant because of its natural immunostimulatory properties. Once administered intramuscularly, mRNA vaccination can activate the adaptive immune system through several pathways:

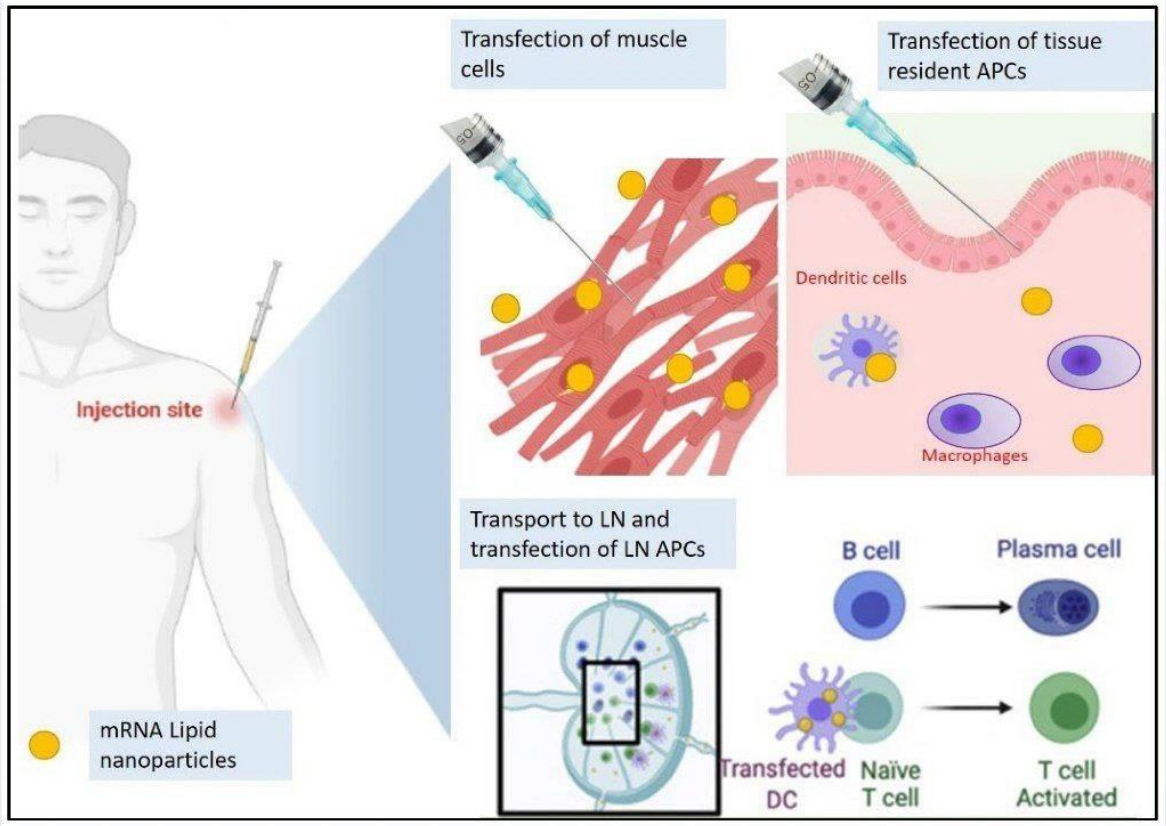
(i) transfection of muscle and skin cells,

(ii) transfection of tissue-resident immune cells such as dendritic cells (DC), macrophages, and Langerhans cells at the injection site, which starts the priming of T and B cells

(iii) transport to secondary lymphoid tissues like lymph nodes (LNs) and the spleen [25].

Figure 4 shows how an intramuscularly administered mRNA-LNP vaccine works. Host cells detect single- stranded RNA (ssRNA) and double-stranded RNA (dsRNA) using various endosomal and cytosolic innate receptors, which are essential for the human innate immune response to external viruses. Toll-like receptors (TLR3 and TLR7) attach to ssRNA in the endosome, while signaling receptors like RIG-I, MDA5, NOD2, and PKR connect to ssRNA and dsRNA in the cytosol. This leads to cell activation and the production of type I interferon and various inflammatory mediators. Type I interferon inhibits cellular translation, which can lower the amount of antigen produced by mRNA vaccines. Currently, available mRNA vaccines use purified IVT mRNA, which is single-stranded and contains modified nucleotides. This reduces binding to TLR3 and TLR7, and immune sensors, thus limiting the overproduction of type I interferon and its suppressive effect on mRNA translation [26]. mRNA vaccines also transfect tissue- resident immune cells, including antigen-presenting cells (APCs) like DCs and macrophages [27].

Figure 4. The site of intramuscular administration and modes of action for mRNA lipid nanoparticles (mRNA-LNPs). mRNA-LNP vaccines can enter muscle cells and also affect the nearby tissue-resident antigen-presenting cells (APCs) at the injection site. Additionally, these vaccines can move into lymph nodes (LNs) and target the LN-resident cells, leading to the activation of T and B cells. Adapted with permission from [25].

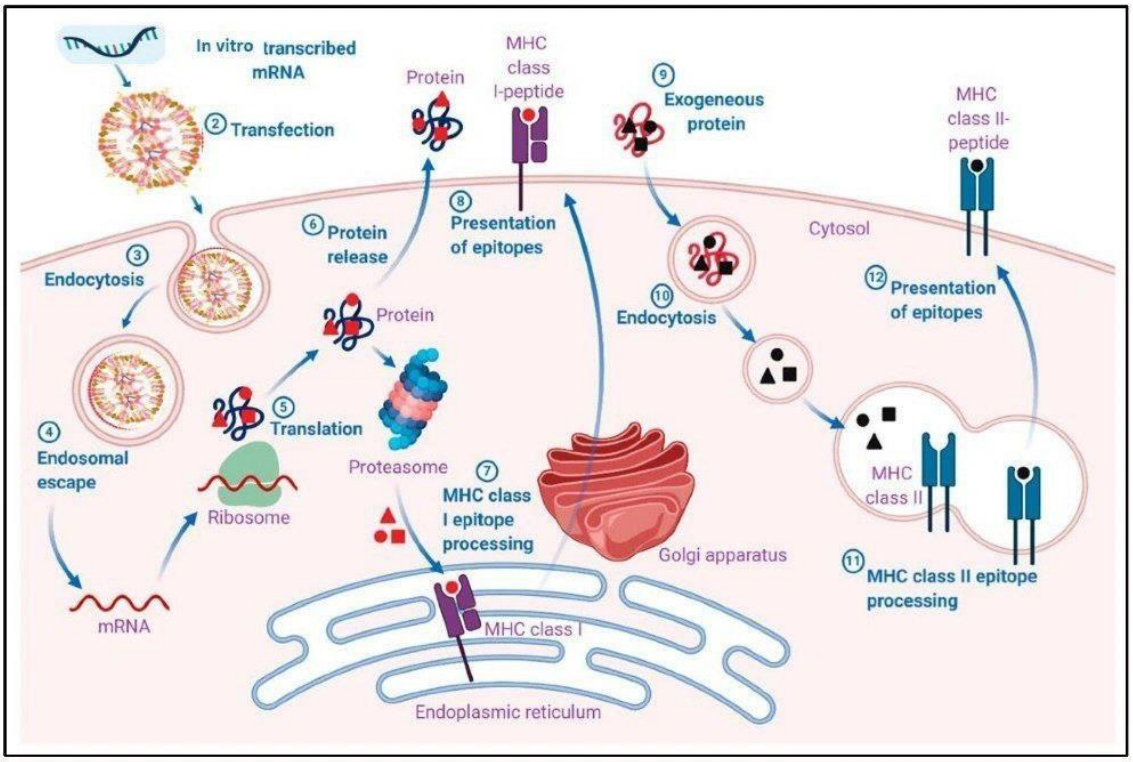


mRNA vaccines work by introducing the mRNA into non-immune cells, leading to the production of the targeted antigen. This antigen is then broken down in the proteasomes within the cytosol. This breakdown reveals the antigenic epitopes, which form a complex with major histocompatibility complex (MHC) class I, presenting them to antigen-presenting cells (APCs) such as cytotoxic T cells that express CD8+. This process helps establish cellular immunity to the antigen produced from the mRNA. When myocytes are transfected by the mRNA vaccines, it can activate bone-marrow-derived dendritic cells (DCs), aiding in the priming of CD8+ T cells.

mRNA vaccines also transfect tissue-resident immune cells, including DCs and macrophages. This action triggers a local immune response at the injection site. The transfection of immune cells with mRNA allows for antigen presentation via MHC class I, which promotes the maturation of CD8+ T cells. Moreover, activating the APCs can also lead to the presentation of the MHC class II pathway, resulting in the activation of T helper cells that express CD4.

After transfecting local immune cells, the response drains into the lymph nodes through the lymphatic system. The lymph nodes contain monocytes and naive T and B cells. Transfection of the lymph node APCs can trigger the priming and activation of both T cells and B cells. Figure 3 illustrates the pharmacological mechanism behind the adaptive immune responses induced by mRNA-LNP vaccines.

Figure 5. Pharmacological mechanism of adaptive immune responses induced by mRNA-LNP vaccines. (1) In vitro transcribed mRNA is enclosed in a lipid nanoparticle (LNP). (2) The mRNA-LNP vaccine enters host cells with the help of specialized lipids on the LNP surface. (3) The cell takes in the mRNA-LNP through endocytosis. (4) After endocytosis, the mRNA escapes from the endosome into the cytosol. (5) The host cell's ribosomes translate the mRNA into the target antigen protein inside the cell. (6) The antigenic protein is either released outside the cell or broken down by a proteasome, revealing the antigenic sites. (7) Major histocompatibility complex I (MHC I) presents the epitope on the cell membrane for antigen presentation (APC). MHC I shows the epitope to CD8+ T cells. (9) The previously released protein can be degraded and presented through MHC II epitopes. B cells recognize the extracellular antigen, which leads to their maturation [25].



3. Drug Delivery Technologies for mRNA Vaccines

This section may be divided by subheadings. It should provide a clear and direct description of the experimental results, their interpretation, and the conclusions that can be drawn. mRNA vaccine molecules are large, ranging from 104 to 106 Da, and carry a negative charge. They cannot cross the lipid bilayer of cell membranes. Naked mRNA would be broken down and destroyed by nucleases in the bloodstream. Additionally, naked mRNA is also captured and engulfed by immune cells in both tissue and serum [31]. Methods to get mRNA molecules into cells include techniques like gene guns, electroporation, and ex vivo transfection. In vivo methods for delivering mRNA involve transfecting immune or non-immune cells with lipids or other transfecting agents [32].

3.1. Lipid Nanoparticles (LNPs)

While naked mRNA, liposomes, and polyplexes have shown clinical effectiveness in humans, LNPs for mRNA vaccines are the only drug delivery system that has proven clinically effective and received approval for human use. The COVID-19 mRNA vaccines against SARS-CoV-2, developed by Moderna and Pfizer/BioNTech, use LNPs to deliver the mRNA payload to the body. LNPs are currently the leading non- viral delivery vector used for gene therapy [33]. Their clinical effectiveness was first

shown when the LNP-siRNA therapeutic Onpattro® (patisiran) was approved by the US FDA for hereditary transthyretin- mediated amyloidosis [34]. LNP formulations represent the most effective and safe method for delivering mRNA vaccines for human immunizations. LNPs provide several benefits for mRNA delivery, including ease of formulation and scale-up, high transfection efficiency, low toxicity, compatibility with different types and sizes of nucleic acids, protection of mRNA from degradation, and extended half-life of mRNA vaccines [35]. LNPs typically include four components: an ionizable cationic lipid, a helper phospholipid, cholesterol, and a PEGylated lipid. These lipids encapsulate the mRNA vaccine's payload and shield the nucleic acid core from degradation [35].

3.2. Cationic and Ionizable Lipids

Cationic lipids were the first type of lipids developed for mRNA vaccine delivery. These lipids contain a quaternary nitrogen atom that gives them a permanent positive charge. This positive charge allows them to form ionic bonds with the negatively charged mRNA vaccines, creating a lipid complex known as a lipoplex [4,36,37]. DOTMA and its synthetic analogue DOTAP were the first cationic lipids used for mRNA vaccines in 1989 [38]. Cationic lipids such as DOTMA, DOPE, and DOGS have been widely employed for mRNA delivery since, including the commercially available Lipofectin, which combines DOPE and DOTMA and was one of the early LNP formulations that succeeded in in vivo translation of mRNA [39]. Early cationic lipids showed promising gene delivery in vitro but lacked effectiveness in vivo. The positive charge from the nitrogen head group and the non-biodegradable nature of these early cationic lipids contributed to their limited delivery capability in live subjects [40]. Ionizable lipids, also known as pH-dependent ionic lipids, represent the second generation of cationic lipids that feature a primary amine, giving them a positive charge at or below physiological pH. These lipids maintain a neutral charge in the bloodstream at physiological pH, improving their safety compared to first-generation cationic lipids. They also extend the circulation time of LNPs compared to those derived from cationic lipids. These advancements came about to address the shortcomings and safety issues of the first-generation cationic lipids, such as immune activation and interaction with serum proteins [33]. DLin-MC3-DMA was the first US FDA-approved ionic lipid used in the siRNA drug Onpattro® [41]. This ionic lipid resulted from modifications to the first ionic lipid, DODMA, by replacing the oleyl tails of DODMA [42,43]. DLinDMA showed better protective immunity against respiratory syncytial virus (RSV) in vivo than DODMA [44]. Researchers further optimized DLinDMA to DLin-KC2-DMA, and then to DLin- MC3-DMA based on several structure-activity relationship studies [45,46]. DLin-MC3-DMA is considered the first generation of ionizable lipids. DLin-MC3-DMA, or MC3, has a long plasma half-life of 72 hours, extending the action duration of siRNA [47]. The MC3 ionizable lipid later proved effective in delivering both mRNA and siRNA [48–54]. However, the long half-life (72 hours) limits the chronic use of vaccines with MC3. Therefore, the next generation of ionizable lipids features biodegradable functional groups that support faster clearance. Adding ester groups improved the biodegradability of MC3 and enhanced its systemic clearance. Ester groups are easy to attach to a lipid, biodegradable, and chemically stable, and they can be cleaved by intracellular esterases. MC3 served as an essential precursor for developing biodegradable ester ionizable lipids [55]. This includes lipids like Moderna's proprietary lipids [56], Acuitas' proprietary lipids [57], and others like YSK12-C4 [58], CL4H6 [59], and L319 lipids, recognized as the second generation of ionizable lipids [47]. Ester-based biodegradable ionizable lipids have shown greater effectiveness in gene delivery compared to the MC3 ionizable lipid. Moderna's lipid 5 was found to have three times the potency, while Acuitas' lipid, ACL-0315 (used in the Pfizer/BioNTech COVID-19 vaccine), had six times the potency compared to MC3 lipid in delivering luciferase mRNA to animals US10166298B2.

The third generation of ionizable lipids is made through an optimized approach, reducing the number of chemical synthesis steps and increasing the high-throughput production of these lipids [60]. 98N12-5 is the first example of a third-generation ionizable lipid [61]. Further modifications and improvements to the 98N12-5 lipidoid led to more advanced analogs, including C12-200 and C14-113 [62,63]. C14-113 lipidoids can specifically target cardiac muscle, which could enhance gene therapies for improving cardiac function [63]. Li et al. identified TT3 as a potent lipidoid for delivering various mRNA molecules encoding CRISPR/Cas9 [64], Factor IX [65], and SARS-CoV-2 [18]. Alongside the interest in improving efficacy, there is also a growing push to enhance the specificity of gene delivery to targeted cells or organs. Targeted delivery for vaccines and immunotherapies to immune cells and lymphoid organs is progressing rapidly. Some targeted agents include lipids with polycyclic tails, such as 11-A-M [66], and those with cyclic imidazole head groups, like 93-O17S [66], specifically designed to reach T cells. Additionally, the cyclic amine head group in lipid A18-Iso5-2DC18 has been shown to bind to the stimulator of interferon genes (STING) protein. This interaction leads to dendritic cell maturation and may provide antitumor effects through immune stimulation [67]. This feature is beneficial for cancer immunotherapy using gene therapy [67]. Gene therapy with third-generation ionizable lipids has also shown potential for treating multidrug-resistant bacterial infections. Cyclic vitamin C-derived ionizable lipids that deliver an anti-microbial peptide and cathepsin B mRNA to macrophages have shown the ability to eliminate multidrug-resistant bacteria and protect mice from bacteria-induced sepsis [68]. LNPs are the most advanced and clinically approved delivery vehicles for mRNA [69].

3.3. PEG-Lipid

Among the components of LNPs, polyethylene glycol (PEG) is a hydrophilic material well known for its many uses in the cosmetic, food, and pharmaceutical industries. The PEGylated lipid component in LNPs is typically linked to an anchoring lipid. PEG is crucial in formulating LNPs, as it reduces the uptake of nanoparticles by filtering organs and improves the colloidal stability of LNPs in biological fluids. This increases the circulation half-life and in vivo distribution of LNPs. Generally, PEG-lipids make up a small percentage of lipid constituents in LNPs (about 1.5%), but they play a vital role in shaping important parameters such as population size, polydispersity index, aggregation reduction, particle stability, and encapsulation efficiency. The molecular weight of PEG and the carbon chain length of the anchored lipid can be adjusted to refine circulation time and immune cell uptake, thus affecting efficiency [70]. Additionally, the PEG-lipid coating on LNPs acts as a hydrophilic barrier, preventing self-assembly and aggregation during storage. Thus, PEG is important for stabilizing LNPs and regulating size by controlling lipid fusion. The amount of PEG inversely affects the size of the LNP; the higher the PEG content, the smaller the LNP [71]. Typically, the molecular weight of PEG ranges from 350 to 3000 Da, while the carbon chain length of the anchored lipid is between 13 and 18 carbons. Various studies indicate that higher molecular weight PEG and longer lipid chains extend the circulation time of nanoparticles and reduce uptake by immune cells. As the PEG-lipid separates from the surface of the LNP, it decreases the circulation time of the LNP, providing more opportunities to deliver the mRNA cargo to target cells, in a phenomenon known as the PEG-Dilemma. In some cases, when the molar percentage of PEG-lipid remains at 1.5%, the in vivo transfection level is independent of the lipid's carbon chain length. An additional advantage of PEG-lipids is their ability to attach a specific ligand to the LNP, facilitating targeted drug delivery [72,73].

3.4. Helper Lipids

Helper lipids play a key role in keeping LNPs stable during storage and while circulating in the body.

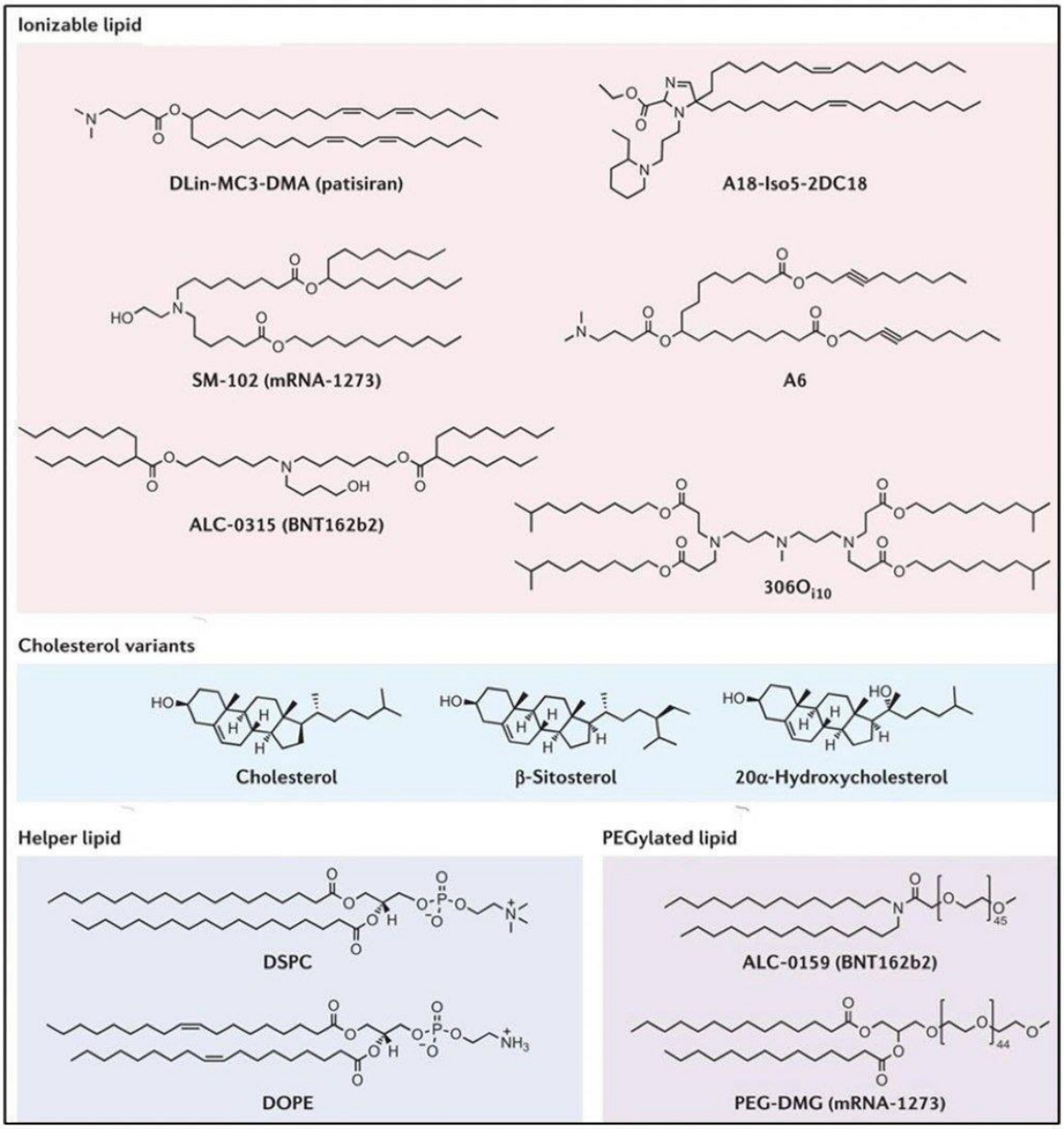
These lipids are glycerolipids and are non-cationic in nature. The most commonly used helper lipids include sterols and phospholipids. Cholesterol is a natural part of cell membranes. It can easily accumulate in the LNP and may be found on the surface, within the lipid bilayer, or even bound to the ionized lipid in the core. Cholesterol is usually added to LNP formulations to improve stability by filling gaps between lipids. It helps regulate the density, uptake, and fluidity of the lipid bilayer matrix within the LNP. This, in turn, controls the rigidity and integrity of the membrane, preventing leaks through the "condensing effect."The hydrophobic tail, sterol ring flexibility, and the polar nature of hydroxy groups in cholesterol have been noted to affect how well LNPs deliver their contents. Cholesterol also helps extend the circulation half-life of LNPs by reducing the amount of protein that binds to the surface. Additionally, it assists in fusing with the endosomal membrane during the cellular uptake of LNPs. Cholesterol is important for lowering the temperature needed for transitioning from the lamellar phase to the hexagonal phase. This allows the mRNA loaded in the LNP to be delivered to the cytosol. Including phospholipids in LNP formulations can enhance encapsulation effectiveness (along with cholesterol) and improve cellular delivery. Typically, the number of phospholipids in LNPs is reduced while the cholesterol content increases to promote longer circulation times. Furthermore, phospholipids boost the trapping efficiency and effectiveness of transfection in LNPs. Research shows that increasing the amount of phospholipids can speed up delivery efficiency. These phospholipids, in their zwitterionic form, are crucial for assembling LNPs by stabilizing the electrostatic interactions among the cationic lipid, mRNA cargo, and surrounding water molecules. Nonetheless, the exact role of phospholipids in delivering mRNA through LNPs remains unclear. Therefore, it is important to explore further how phospholipids enhance particle stability and efficacy in vivo. Figure 4 illustrates the components of LNPs, including ionizable lipids, cholesterol, helper lipids, and PEGylated lipids.

3.5. Physicochemical Properties Affecting mRNA-LNPs

LNPs have many unique features, most of which are beneficial; however, a few can cause unwanted toxic effects. Hence, it is important to understand the physicochemical properties that impact mRNA-loaded LNPs.

Size and Surface Area: The size and surface area determine how LNPs interact with biological systems, as well as their distribution, elimination, internalization, degradation, and response. Smaller size leads to a larger surface area, making LNPs more reactive to their environment. Key biological processes, including endocytosis and cellular uptake, are largely influenced by particle size. Size-dependent toxicity arises from LNPs' ability to enter biological systems and alter macromolecules, changing essential biological functions. For vaccines, efficient delivery has been observed while maintaining a particle size of about 50 nm, regardless of chemical composition.

Figure 4. Components of lipid nanoparticles including ionizable lipids, cholesterol, helper lipids, and PEGylated lipids [35].



4. mRNA Vaccines Manufacturing

mRNA vaccines have several advantages over traditional vaccines, such as easier development, simpler scaling, and faster production. Like other vaccines, mRNA vaccine products go through three main steps in their manufacturing: upstream production, downstream purification, and mRNA drug formulation. This section will cover those steps and recent developments aimed at improving mRNA vaccine production.

4.1. Upstream Production

The upstream production of mRNA vaccines involves creating the mRNA transcript from a plasmid that contains the gene of interest. This process is called the in vitro transcription reaction (IVT). The IVT enzymatic reaction depends on RNA polymerase enzymes like T7, SP6, or T3. These enzymes help synthesize the target mRNA from a linearized DNA template with the gene of interest. A linearized DNA template is made by cutting a plasmid with restriction endonucleases or by amplifying the gene through PCR, which can also produce mRNA molecules. The key enzymes in an IVT reaction include: (i) RNA polymerase, which converts DNA to RNA, (ii) inorganic pyrophosphatase (IPP), which increases IVT reaction yield, (iii) guanylyl transferase, which adds GMP nucleoside to the 5' end of mRNA, (iv) Cap 20- O-Methyltransferase (SAM), which adds a methyl group at the 20 position of the 5' cap, (v) DNase I, which removes contaminating genomic DNA from RNA samples and breaks down DNA templates in the IVT reaction, (vi) poly(A) tail polymerase, and (vii) modified and unmodified nucleoside triphosphates (NTPs). These enzymes help develop the mRNA transcript from the plasmid with the gene of interest. Capping enzymes like SAM and guanylyl transferase form a 5' cap at the mRNA's 5' end, while the poly(A) tail polymerase creates the poly(A) tail. Another approach for 5' capping is the co-transcriptional method, where the 5' cap is prepared first and added to the mRNA without an enzyme. This co- transcription process can use CleanCap® Reagent AG.

4.2. Downstream Purification

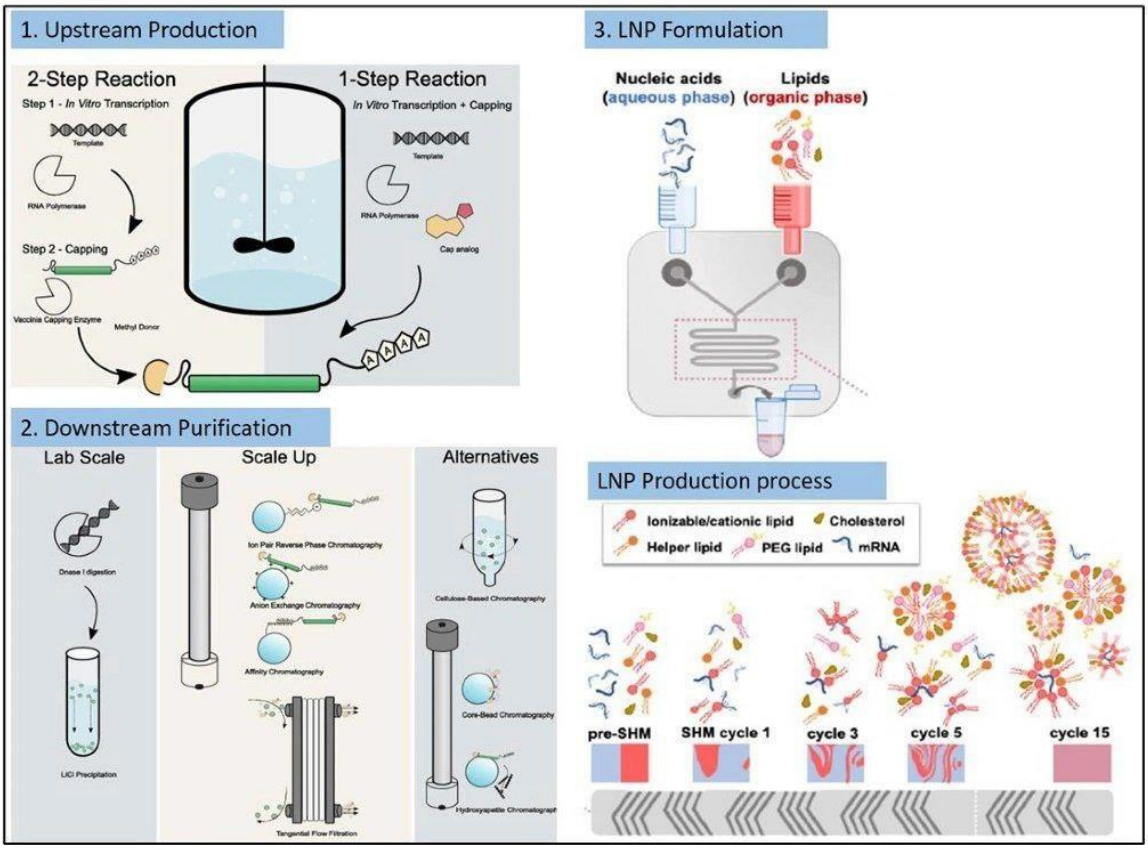
After the IVT reaction produces mRNA in the upstream production phase, the mRNA is isolated and purified through several steps in downstream processing. The IVT mixture includes impurities such as leftover NTPs, enzymes, improperly formed mRNAs, and DNA plasmid templates. Purifying IVT mRNA at the lab scale often uses methods that remove DNA through DNase enzyme digestion followed by lithium chloride (LiCl) precipitation. However, these lab methods do not completely eliminate aberrant mRNA species, such as dsRNA and truncated RNA fragments. Removing these impurities is crucial to achieving a pure mRNA product that can deliver its intended efficacy and safety. Ineffective purification can lead to an mRNA vaccine with lower translation efficiency and unwanted immune responses. For instance, a 10–1000-fold increase in mRNA transfection and related protein production was seen when modified mRNA was purified through reverse-phase HPLC before being delivered to dendritic cells. Chromatography is a widely accepted purification process in the biopharmaceutical industry for vaccines and biologic drug products. The first large-scale procedure for purifying RNA oligonucleotides using size exclusion chromatography (SEC) was published in 2004. SEC has advantages like selectivity, scalability, versatility, cost-effectiveness, and high purity and yield for nucleic acid products. However, SEC cannot

remove impurities of similar sizes, such as dsDNA. Instead of SEC, ion-pair reverse-phase chromatography (IEC) has proven to be a great purification method for mRNA vaccines. IEC efficiently separates target mRNA from IVT impurities by using the charge differences between the target mRNA and the impurities. IEC offers many benefits, including the ability to separate longer RNA transcripts from the target, high binding capacity, cost-effectiveness, and scalability. Because IEC operates under denaturing conditions, the process can be complex and sensitive to temperature.Affinity-based chromatographic separation is another method for purifying mRNA. Deoxythymidine (dT)-Oligo dT is a sequence that binds to the poly(A) tail of the mRNA. Chromatographic beads containing Oligo dT can help purify mRNA vaccines. Tangential flow filtration (TFF) or core bead filtration can be used to remove smaller impurities. As a final polishing step, hydrophobic interaction chromatography (HIC) linked to a connective interaction media monolith (CIM) column may be very useful.

4.3. Formulation

Since mRNA molecules are negatively charged, they need to be formulated in a lipid-based drug delivery system to prevent degradation and improve transfection efficiency and half-life. LNPs are the most reliable lipid-based non-viral carriers approved by the US FDA for delivering mRNA vaccine substances. mRNA LNPs form by precipitating lipids dissolved in an organic phase and mixing them with mRNA in an aqueous phase. Commonly used lipids in the organic phase include ionizable lipids, cholesterol, helper lipids, and PEG-lipids. The mRNA is dissolved in a citrate or acetate buffer at pH 4. When mixing the aqueous and non-aqueous solutions, the ionizable lipid gets protonated, creating an electrostatic attraction between the protonated lipid and the anionic mRNA. This interaction works with the hydrophobic interactions of other lipids and leads to the self-assembly of mRNA-LNPs with the mRNA enclosed within the nanoparticles. This process is called microprecipitation. After forming LNPs, they are dialyzed to eliminate the non-aqueous solvent, usually ethanol, and raise the solution pH to a physiological level. Microfluidic mixers help create small-sized LNPs with a low polydispersity index and high mRNA encapsulation efficiency. Microfluidic mixing is the most common method for formulating mRNA LNPs at both lab and GMP levels. Precision NanoSystems' NanoAssemblr® platform has been widely used for developing and producing LNP formulations in controlled environments. This system features a staggered herringbone micromixer (SHM) cartridge design. The SHM structure allows the mixing of two solvents in microseconds, which is faster than what is needed for lipid aggregation, thus producing small nanoparticles of uniform size. The settings on the NanoAssemblr® can be easily adjusted to change the flow rate and volume of the aqueous and non-aqueous phases to obtain LNPs of the desired size and distribution. A flow rate of 12–14 mL/min and a volume ratio of 3:1 for non- aqueous to aqueous phase is typically used to make small monodisperse LNPs. Despite the advantages of SHMs for LNP production, their use in GMP manufacturing is limited due to solvent incompatibility. Prolonged exposure of the SHM and its internal parts made of polydimethylsiloxane to ethanol can damage them, complicating cartridge replacement during continuous GMP production. Therefore, T- mixers are used for LNP scaling and manufacturing. They can produce LNPs similar to those made with SHMs, handle higher flow rates and volumes (60–80 mL/min), and are compatible with organic solvents like ethanol. Figure 5 illustrates the mRNA vaccine manufacturing processes.

Figure 5 shows the steps and phases of the manufacturing process for mRNA vaccines. The mRNA manufacturing process is divided into three phases: upstream mRNA production, downstream mRNA purification, and formulation of mRNA lipid nanoparticles. In principle, mRNA could be made by means of a one-step co-transcriptional reaction with capping agent, or two-step reaction whereby capping is performed enzymatically. Purification of mRNA at laboratory scale consists of a DNase I digestion enzyme and a subsequent LiCl precipitation of mRNA. At commercial scale mRNA purification utilizes well-established chromatographic techniques with tangential flow filtration (TFF). Lastly, the formulation of the mRNA vaccines is accomplished when mRNA aqueous solution is added or mixed with fatty lipids in a non-aqueous phase, which promotes self-assembly of lipid nanoparticles (LNPs) encasing mRNA core, which is negatively charged. The mixing of mRNA and lipid in the staggered herringbone micromixer (SHM) and the assembly procedures occurs in different cycles that result in the made final mRNA-LNP vaccines. Adapted with permission from [86,90].



5. {5.1} mRNA-1345

mRNA-1345 is a vaccine candidate that Moderna developed for respiratory syncytial virus (RSV) infection. It encodes an RSV protein called prefusion F glycoprotein, which triggers an effective neutralizing antibody response. This protein plays a key role in how the virus enters cells and spreads from one cell to another. It is essential for the spread of RSV infection.This vaccine is based on lipid nanoparticles and includes improved protein and codon sequences. The US FDA has recently given mRNA-1345 a fast-track review designation for adults older than 60 years. Previous vaccines for RSV infection have failed in clinical trials due to low immune responses. Moderna has recently shared interim results from an ongoing Phase 1 study. This study focuses on the tolerability, side effects, and immune response of mRNA-1345 in children, young adults, older adults, and women who could become pregnant. Results indicate that the vaccine was well tolerated at all dose levels as of the data cut-off date. The study is expected to finish in 2023. A Phase 2/3 study of the mRNA-1345 vaccine in adults aged 60 and over is underway. This study aims to assess the safety and tolerability of the vaccine and to show how effective a single dose of mRNA-1345 is in preventing the first episode of RSV-associated lower respiratory tract disease (RSV-LRTD) compared to a placebo from 14 days after the injection up to 12 months. The study will be done in two placebo-controlled phases: Phase 2 will involve 400 to 2000 participants, and Phase 3 will include over 30,000 participants. The main goal is to assess the vaccine's safety and effectiveness. Safety measures include monitoring participants for the occurrence of adverse reactions, adverse events, serious adverse events, and adverse events of particular interest. The primary effectiveness measure is the Vaccine Efficacy (VE) of mRNA-1345 in preventing the first occurrence of RSV-LRTD from 14 days after the injection through 12 months. This study began in November 2021 and is expected to complete by November 2024.

Table 1. Active and completed clinical trials of mRNA vaccines in the treatment of viral infections. Adapted from Wang et al. 2021.27

Infection	Phase	Status	Drug treatment	NCT number
SARS-CoV-2	I	Active	BNT162b1 + placebo	NCT04523571
	I	Active	CVnCoV vaccine + placebo	NCT04449276
	III	Active	mRNA-1273 + placebo	NCT04470427
	I/II/III	Recruiting	BNT162b1 + BNT162b2	NCT04368728
	II	Active	CVnCoV	NCT04515147
	I	Active	mRNA-1273	NCT04283461
	II	Active	mRNA-1273 + placebo	NCT04405076
	II	Not yet recruiting	2 doses of BNT162b2 or one dose of BNT162b2s01	NCT04949490
Rabies	I	Completed	CV7201 mRNA encoding the rabies virus glycoprotein	NCT02241135
	I	Active	Rabipur®	NCT03713086
HIV-1	I/II	Completed	mRNA-transfected autologous DCs+/−autologous DCs with no mRNA transfection	NCT00833781
	I	Terminated	TriMix mRNA+/−HIV mRNA	NCT02413645
	II	Completed	iHIVARNA-01 + TriMix+/−Placebo	NCT02888756
Zika virus	I	Completed	mRNA-1325 + placebo	NCT03014089
	I	Active	mRNA-1893 + placebo	NCT04064905
Tuberculosis	I	Completed	GSK 692342	NCT01669096
Ebola virus	I	Completed	two separate mRNAs encoding two Zaire strain Ebola glycoproteins, respectively	NCT02485912
Influenza	I	Completed	VAL-506440 + placebo	NCT03076385
	I/II	Recruiting	mRNA-1010 + placebo	NCT04956575
Cytomegalovirus	I	Completed	mRNA-1647 + placebo	NCT03382405
	II	Recruiting	mRNA-1647 + placebo	NCT04232280
Respiratory syncytial virus (RSV) vaccine	I	Recruiting	mRNA-1345 + placebo	NCT04528719
Human Metapneumovirus and Human Parainfluenza Infection	I	Completed	mRNA-1653 + placebo	NCT03392389
	I	Recruiting	mRNA-1653 + placebo	NCT04144348

Table 2. Active and completed clinical trials of mRNA vaccines in the treatment of solid malignant tumors. Adapted from Wang et al. 2021.27

Tumor type	Phase	Status	Drug treatment	NCT number
Non-small cell lung cancer (NSCLC)	I/II	Recruiting	BI 1361849 (CV9202) + Durvalumab+/−Tremelimumab	NCT03164772
	I/II	Recruiting	Personalized mRNA vaccine encoding neoantigen	NCT03908671
	I/II	Recruiting	Suppressor of cytokine signaling (SOCS) 1, MUC1 and Survivin mRNA-loaded DC + cytokine-induced killer cells	NCT0268868
Melanoma	I	Completed	mRNA+GM-CSF	NCT00204607
	I/II	Completed	DCs transfected with hTERT, survivin and p53	NCT00978913
	I	Completed	Dendritic cells electroporated with mRNA encoding gp100 and tyrosinase	NCT00940004
	I/II	Completed	TriMix-DC	NCT01066390
	I	Completed	DCs loaded with mRNA encoding tumor-associated antigens gp100 and tyrosinase+/ −cisplatinum	NCT02285413
	I/II	Completed	mRNA coding for melanoma associated antigens+GM-	NCT00204516
	I	Completed	CSF mRNA-transfected DCs + IL-2	NCT01278940
	I	Completed	autologous dendritic cell vaccine by mRNA	NCT01530698
	II	Completed	Electroporation Autologous dendritic cell vaccine	NCT00243529
	I/II	Recruiting	mRNA-4157 + pembrolizumab	NCT03897881
	I	Active	Autologous Langerhans-type dendritic cells electroporated with mRNA encoding a tumor-associated antigen	NCT01456104
	II	Active	Lipo-MERIT	NCT02410733
	I	Terminated	Dendritic cells - transfected with hTERT-, survivin- and tumor cell-derived mRNA+ex vivo T cell expansion and reinfusion+Temozolomide	NCT00961844
	I	Terminated	(NCI)-4650, an mRNA-based, personalized cancer	NCT03480152
	I/II	Terminated	vaccine Autologous dendritic cells with mRNA	NCT0092901
Ovarian cancer	I	Recruiting	W_ova1 + carboplatin/paclitaxel	NCT04163094
	I/II	Terminated	DC-006 vaccine	NCT01334047
	I	Terminated	DCs loaded with TERT-mRNA and survivin-peptide	NCT01456065
Prostate cancer	I	Completed	mRNA-transfected dendritic cells	NCT01278914
	II	Completed	DCs transfected with PSA, PAP, survivin and hTERT mRNA+docetaxel	NCT01446731
	I/II	Completed	DC loaded with protamine/mRNA encoding keyhole limpet hemocyanin (KLH) + DC loading with MHC I binding peptides, NY-ESO-1 and MUC1 PepTivator®	NCT02692976
	I	Active	Dendritic cell vaccine	NCT01197625
	II	Withdrawn	Human telomerase reverse transcriptase mRNA (hTERT mRNA) transfected dendritic cell	NCT01153113
	II	Terminated	CV9104	NCT02140138
	I/II	Unknown	Peptide vaccine+montanide ISA-51+/−GM-CSF+/− imiquimod+/−mRNA/protamin	NCT02452307
Gastrointestinal cancer	I/II	Completed	CEA mRNA-loaded DCs	NCT00228189
	I/II	Completed	Personalized mRNA vaccine encoding neoantigen	NCT03468244
	I/II	Recruiting	Adenovirus-transfected autologous DCs + CIK cells	NCT02693236

5.2. mRNA-1010

mRNA-1010 is a quadrivalent vaccine candidate developed by Moderna for flu. It includes the hemagglutinin (HA) protein from four seasonal influenza viruses based on World Health Organization recommendations. These viruses are seasonal influenza A/H1N1, A/H3N2, and influenza B/Yamagata and B/Victoria lineages. HA is important for vaccine development because it provides broad protection against influenza and is the main target of current influenza vaccines. The efficacy of mRNA-1010 has been assessed in Phase 1 and Phase 2 studies. In December 2021, Moderna released interim results of the ongoing Phase 1 study, which evaluated mRNA-1010 at three doses: 50 µg, 100 µg, and 200 µg in younger and older adults. Results showed that mRNA-1010 increased hemagglutination inhibition assay geometric mean titers against all strains 29 days after vaccination at all doses in all participants, with no significant safety findings. The company also confirmed that the ongoing Phase 2 study with mRNA-1010 has reached full enrollment, and an interim analysis is planned for 2022. A Phase 3 active-controlled study (NCT05415462) is underway to assess the immunogenicity and safety of the mRNA-1010 seasonal influenza vaccine in adults aged 18 and older. The active comparator is any licensed quadrivalent inactivated seasonal influenza vaccine. The primary goals of this study are to evaluate the humoral immunogenicity of mRNA-1010 compared to that of an active comparator against vaccine-matched influenza A and B strains at Day 29, and to assess the safety and reactogenicity of mRNA-1010. Safety endpoints include monitoring for adverse reactions, adverse events, serious adverse events, and adverse events of special interest. Primary efficacy endpoints are geometric mean titer (GMT) of anti- hemagglutinin (HA) antibodies at Day 29 and the percentage of participants achieving seroconversion. This study started in June 2022 and is expected to be completed by August 2023 NCT05415462.

5.3. mRNA-1647

mRNA-1647 is a vaccine candidate developed by Moderna for cytomegalovirus (CMV) infection in women of childbearing age. It consists of six mRNAs that encode two antigens on the surface of CMV. Five mRNAs encode the subunits that make up the membrane-bound pentamer complex, while the sixth encodes the full-length membrane-bound glycoprotein B (gB). The mRNA-1647 vaccine instructs human cells to produce the antigens, resulting in functional antigens similar to those presented to the immune system by CMV during a natural infection. So far, the mRNA-1647 vaccine has been evaluated in Phase 1 and Phase 2 studies. Interim analysis results from these studies were positive and led to the start of a Phase 3 study to confirm the efficacy and safety of mRNA-1647. This Phase 3 study (NCT05085366) is a randomized, observer-blind, placebo-controlled study that evaluates the efficacy, safety, and immunogenicity of the mRNA-1647 vaccine in healthy participants aged 16 to 40 years. The primary objective of the study is to assess the efficacy of the mRNA-1647 vaccine in CMV-seronegative female participants and to evaluate the safety and reactogenicity of the mRNA-1647 vaccine in all participants. Safety endpoints include monitoring for adverse reactions, adverse events, serious adverse events, and adverse events of special interest. Primary efficacy endpoints include seroconversion from a negative to a positive result for serum immunoglobulin G (IgG) against antigens not encoded by mRNA-1647, with the timeframe from Day 197 (28 days after the third injection) to Day 887 (24 months after the third injection). The study began in October 2021 and is expected to finish by July 2025 NCT05085366.

5.4. Clinical Safety of mRNA-Based Vaccines

The main goal of early-stage clinical trials for any vaccine candidate is to assess its safety in humans. Safety is monitored throughout the clinical development process by tracking adverse events, deaths, laboratory findings, and other factors.A vaccine can only receive marketing authorization if its safety profile is deemed

acceptable. Regulatory agencies and Institutional Ethics Committees (IEC) can halt the clinical trial if any concerning events occur. Adverse events related to the vaccine candidate are expected to resolve quickly. Even after vaccines receive marketing approval, sponsors must keep monitoring the safety profile. Toxicity is a key concern for mRNA-based vaccines due to the presence of nucleosides. Literature reports that the toxicity of some nucleoside-based anti-cancer and antiviral drugs can be traced to unnatural nucleosides. Specifically for mRNA vaccines, hepatotoxicity was the most common toxicity noted in preclinical studies for a vaccine under development for Crigler–Najjar syndrome. This may be linked to any toxic excipients used in formulating the lipid nanoparticles for delivery. In another study involving an mRNA vaccine for rabies, systemic adverse events were observed during a clinical trial due to the inflammatory nature of mRNA. Most toxicities related to mRNA vaccines stem from the excipients used in formulation or other solvents used during development. These toxicities can be minimized by using excipients within safety limits and following processes that reduce residual toxic components in the vaccine. In the future, possible toxicities of mRNA-based vaccines may include local and systemic inflammation, biodistribution and persistence of the expressed immunogen,

stimulation of auto-reactive antibodies, and potential toxic effects of non-native nucleotides and delivery system components. Additionally, these vaccines could induce strong type I interferon responses, edema from extracellular naked RNA, blood coagulation, and pathological thrombus formation. On a positive note, several mRNA vaccines have received approval for human use from global health authorities. All approved vaccines showed acceptable safety profiles during clinical trial evaluations. For example, the two COVID-19 mRNA vaccines, Pfizer–BioNTech (Comirnaty) and Moderna (Spikevax), have demonstrated excellent safety and efficacy. Overall, the safety profiles of many mRNA- based vaccines in clinical development have been acceptable (well tolerated), and very few, if any, have been withdrawn from clinical trials to date. Most adverse events reported during clinical studies involve injection site reactions. Sponsors must prioritize safety during vaccine development by conducting thorough toxicity testing in nonclinical studies. Observations from nonclinical studies should be taken into account during clinical trials and monitored carefully.

6. mRNA purification and quality control

Purifying and controlling the quality of mRNA are key steps in ensuring the effectiveness, stability, and safety of mRNA vaccines. After in vitro transcription (IVT), mRNA products often contain impurities like DNA templates, enzymes, and double-stranded RNA (dsRNA) byproducts. These impurities can undermine stability and trigger unwanted immune responses [113]. Various purification methods are available, including size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC), reverse-phase high-performance liquid chromatography (RP-HPLC), and affinity-based techniques such as oligo-dT columns. Notably, RP-HPLC has effectively removed dsRNA impurities, increasing mRNA translation efficiency by up to 1000-fold [65]. New enzymatic treatments and multimodal chromatographic methods are also appearing to further improve purity and scalability [114]. High- efficiency purification not only meets regulatory standards but also sets the stage for future improvements in mRNA vaccine technology. This enables the consistent production of high-purity mRNAs for next-generation vaccines and therapies. This is especially important as mRNA vaccines branch out from infectious diseases to address non-communicable diseases like cancer and rare genetic disorders, where accuracy and reliability are essential.

7. Conclusions

Decades of development and research in mRNA design and delivery technology have made mRNA vaccines a remarkable tool for fighting pandemics and existing infectious diseases. The first two mRNA vaccines against SARS-CoV-2 were created at an unexpected pace. These vaccines have surpassed expectations and established a strong foundation for the future of mRNA vaccines. It is clear from numerous clinical trials that mRNA vaccines can compete with or even replace traditional vaccine platforms soon. mRNA technology could lead to more effective vaccines against difficult pathogens and provide treatments for various cancers in the near future. However, improvements in mRNA delivery technologies will be necessary for safer, more effective, and cold-chain-free mRNA vaccines that can vaccinate billions of people worldwide. Further research is needed to understand how mRNA vaccines affect innate immune responses. The wealth of positive safety and efficacy data for the approved mRNA vaccines, along with a proven pathway for regulatory approval, gives hope to the scientific community that mRNA treatments have great potential to change modern approaches to vaccination, protein replacement therapy, and cancer immunotherapy[35].

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