# The Advanced Development of mRNA Vaccines

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**Abstract:** There are many viruses in the living environment of human beings today. Once these viruses infect humans, they will begin to spread rapidly, and the cure rate of infected people is also variable, mainly depending on the patient's physical condition. At the same time, due to the incubation period of the disease, once the virus begins to spread, the consequences are very serious. From previous smallpox, polio, to modern SARS, MERS, and the new crown, the virus has brought losses to humans every time. So far, the most effective way for humans to fight the virus is to vaccinate. Since the eighteenth century, humans have invented many kinds of vaccines, including live-attenuated vaccine, inactivated vaccine, carrier vaccine, nucleic acid vaccine, and so on. At present, inactivated vaccines and attenuated vaccines are the most commonly used and most mature technologies. However, the development center in recent years has shifted to nucleic acid vaccines, especially mRNA vaccines. The mRNA vaccine itself has shown its advantages in the treatment of tumors. After the epidemic of the new coronavirus, its various advantages have allowed it to surpass traditional vaccines. This article will focus on some of the characteristics of mRNA, the working mechanism of mRNA, the characteristics of some types of mRNA, the relationship between mRNA vaccines and the new crown, and the prospects of mRNA.

## 1. Introduction

This vaccine-like method of protecting against viruses first appeared in England in the eighteenth century. At that time, it was accidentally discovered that people who had been infected with vaccinia would not be infected by the smallpox epidemic at the time. Although humans did not have the knowledge of the immune system at that time, what is certain is that the human body can be induced and become resistant to the virus before being infected. In 1796, the British doctor Edward Jenner invented the first vaccine in human history, the vaccinia vaccine. Then, French Pasteur invented the cholera vaccine and the rabies vaccine, and this marks the appearance of Inactivated and attenuated vaccines.

Since then, with the rapid development of molecular biotechnology, biochemistry, genetics, and immunology, the theoretical basis and technical level of vaccine development have been continuously improved and improved. Some traditional classic vaccine varieties have been further transformed into new vaccines, while others have been used. Vaccines that cannot be developed by classical technology have found a way to solve the problem. Therefore, subunit vaccines and recombinant vaccines for different infectious and non-infectious diseases were born. Vaccine development experience during the H1N1 influenza pandemic in 2009 showed that the speed of vaccine development at that time was not enough to deal with sudden pandemic events. To accelerate the speed of vaccine development, scientists from Novartis cooperated with researchers from the J. Craig Venter Institute and Synthetic Genomics to explore ways to use synthetic biology to rapidly generate candidate vaccines from genetic sequence data.

2020 With the COVID-19 epidemic sweeping the world, the rapid development of the new crown vaccine is undoubtedly a highlight of the multiple efforts in the field of biomedicine to respond to COVID-19. A few days ago, BNT162b2 developed by Pfizer/BioNTech and mRNA-1273 developed by Moderna have successively obtained emergency use authorization (EUA) granted by the FDA. Both of them have reached a protective effect of about 95%, bringing hope to the progress of curbing the

COVID-19 epidemic. These two vaccines also represent the first use of mRNA vaccine technology in a wide range of populations.

## 2. Mechanisms of Immune System and mRNA

### 2.1 Transcription

Normally, mRNA will be transcribed in the cell, and no RNA is an intermediate product of the cell's protein production. The mRNA produced by the human body comes from the DNA in our cell nucleus, which contains the genetic information of individual humans, and the information is expressed in the form of nucleotides. There is also an organelle called RNA polymerase in the nucleus of the cell. In mRNA polymerase, each nucleotide in DNA is transcribed into another according to the principle of base complementary pairing. Each transcribed nucleotide then constitutes mRNA fragments.

### 2.2mRNA Processing

The name of the direct product after transcription is actually pre-mRNA. At this stage, there are some fragments of mRNA that cannot be directly used for translation. In pre-mRNA, segments that can be translated (exons, containing codons) and non-translatable segments (intron, usually regulatory sequences) appear alternately. [1-2] Therefore, to obtain a complete sequence that can directly produce a protein (mature mRNA), the shearing and processing of pre-mRNA is essential. This process is called alternative splicing.

### 2.3 Translation

The mature mRNA passes through the nuclear membrane to the rough endoplasmic reticulum. The next step is translation, which is done by the ribosome in rER. The three codons on mature RNA form a group, corresponding to different amino acids, which are carried by tRNA to the subunits of the ribosome. From the 5'to 3'direction, the protein has formed its primary structure. Then, these proteins will enter the Golgi apparatus for modification and folding, and then send them out of the cell.

### 2.4 Vaccines and Immune Response

The immune system can be divided into the innate immune system and adaptive immune system. The characteristics of the innate system are mainly reflected in the protection of some physical attacks. And there is no target specificity. The innate immune system includes such physiologic barriers as the normal body temperature, fever, gastric acidity,

lysozyme, interferon, and collectins.[3] Compared with the innate immune system, the advantage of the adaptive immune system is more obvious, which is reflected in the antibodies that do not attack all indiscriminately but target different individuals. This also created its second characteristic, the memory of the pathogen. There are many different immune cells involved in the immune response triggered by the adaptive immune system, and the mechanism is quite complicated. After the first long immune response, the body can quickly activate the immune response the next time it sees the pathogen.

The nature of the corresponding antigens is an immune response-inducing antigen.[4] Its essence is that a eukaryotic expression vector containing pathogen antigen genes can be taken up by body cells and express pathogen antigen protein when it is introduced into the body, thereby inducing the body's immune response to the protein. Depending on the route and site of introduction, it can trigger a systemic or local immune response. In the systemic immune response, both humoral immunity and cellular immunity can be induced.

The antigen is presented to the immune system in several ways:

(1)The antigen is processed in the cell and combined with MHC I molecules and presented to the cell surface to stimulate cytotoxic T lymphocytes (CTL).

(2)The protein is released from the cell and binds to the B cell receptor, Stimulate B cells.

(3) Part of the released protein is absorbed and degraded by antigen-presenting cells, and then combined with MHCII molecules to stimulate helper T cells, which finally triggers a response from

the immune system. The response level of the immune system is related to different immune parts, the expression level of cells, and whether or not to increase immune regulatory genes.

## 3. Design of mRNA Vaccines

### 3.1 5’cap

5'cap is closely related to the stability of mRNA. The 5‘ cap can eliminate free phosphate groups in the mRNA sequence to significantly enhance the stability of mRNA, which allows the ribosome to recognize the beginning of mRNA and improves translation efficiency by binding to the eukaryotic translation initiation factor 4E (eIF4E)[5]. At the same time, because the mRNA base sequence is arranged from 5'to 3', 5'cap has a more basic function, which is to ensure that the translation sequence is correct. This is a crucial step for the efficiency of mRNA vaccines.

### 3.2 Poly A tail

Poly-A tail is the most important factor for mRNA stability besides 5'cap. The main reason is that most of the degradation of mRNA starts from the Poly-A tail, which is the 3' tails of different lengths have different effects on the efficiency and stability of mRNA translation.

Moreover, Poly (A) binding protein (PABP) can link to the 5’ Cap through translational initiation factors, such as eIF4G and eIF4E which in turn affects the closed-loop structure of mRNA and synergistically regulates the stability and translation efficiency of mRNA.[6]

### 3.3 3’UTR and 5’UTR

5'UTR and 3'UTR are factors that affect translation efficiency, because these regions correspond to the regulatory part of the mRNA sequence. For 5'UTR, the main function is to ensure that there is no error in the starting position of the translation.5'UTRs that are too long or arranged too tightly may hinder the subsequent translation process. Avoiding the gene sequence in 5‘UTR, which is identical to the upstream of ORF, can effectively prevent false start and replacement of the reading frame during mRNA translation.[7] One minimalistic 5'-UTR, consisting of 14 nucleotides combining the T7 promoter with a Kozak consensus sequence, yielded similar or even higher expression than a 37 nucleotides human alpha globin 5'-UTR containing mRNA in HepG2 and A549 cells.[8]

3'UTR is slightly different from 5'UTR. It appears at the end of the sequence just like the poly-A tail, causing it to have a significant impact on the stability of mRNA, repeated coden or wrong stop coden often appear at the end. Effective modification of 3'UTR can significantly improve the stability of mRNA. According to Alexandra G. Orlandini von Niessen, An AES-mtRNR1- and mtRNR1-AES-based 30 UTR system discovered here is useful for carrying out mRNA-based genetic transfer, as demonstrated by mRNA-based vaccination and reprogramming of human fibroblasts.[9]

## 4. Delivery of mRNA Vaccines

The delivery of mRNA is a prerequisite for translation and is very challenging. It is mainly reflected in allowing mRNA to pass through the cell membrane and ensuring that the mRNA will not be degraded. There are a large number of mRNA enzymes in the blood tissue of the human body, which constitutes a great obstacle. Secondly, as a large molecule, it is difficult for mRNA to pass through the phospholipid bilayer. In addition, the mRNA molecule is negatively charged with the phospholipid bilayer.Taken together, a carrier is crucial for the delivery of mRNA.

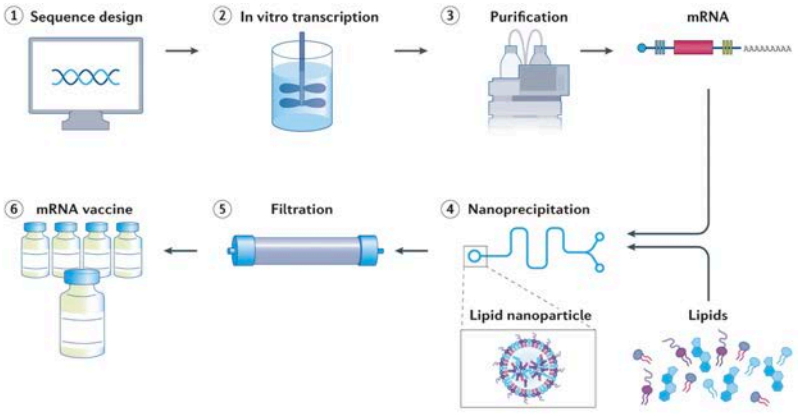


**Figure 1.** Design and Modification of mRNA Vaccines

### 4.1 Nanolipids

Positively-charged LNPs have characteristics such as polyethylene glycol, phospholipids, and cholesterol, with amino lipids that can be ionized.[10] Ionizable amino lipids assist mRNA in escaping the endosome by interacting with lipids on the membrane of the endosome.[11-12] In addition to polyethylene glycol, phospholipids and cholesterol can stabilize LNP structure integration. Moreover, polyethylene glycol spatially hinders mRNA binding and plasma protein binding, which accelerates clearance by reticuloendothelial (ER). Once they escape endosomes, LNPs release mRNA into the cytoplasm to synthesize vaccine antigens.

### 4.2 Polymers

By condensing nucleic acids into nanoplexes, cationic polymers shuttle nucleic acids across cellular membranes and subcellular membranes.Catalytic polymers of both linear and branched structure can be used for endocytosis and subsequent endosomal escape.[13]

## 5. Different types of mRNA vaccine

### 5.1 Self-amplifying mRNA vaccine

SaRNA is much larger than non-amplified mRNA. They contain the basic elements of mRNA (cap, 5'UTR, 3'UTR, and poly(A) tail)Variable length) but a large open reading frame (ORF) at 5', end encoding, four non-structural proteins (nsP1-4), and a subgenomic promoter

Currently, common self-amplifying mRNA (SAM) vaccines, are based on the alphavirus genome, in which the RNA replication machinery is maintained, but structural proteins encoded by the genes are replaced with target antigens.[12] When given alone or in a trivalent combination, sa-RNA vaccines protect against influenza A or B infection. sa-RNA vaccinations provided protection with a 64-fold lower dosage than non-amplifying synthetic mRNA vaccines, perhaps due to extended and enhanced transgene expression.[14]

Dendritic cell mRNA vaccines

Dendritic cell mRNA vaccines usually focus on the tumor.

The utilization of the patient's own DCs loaded with antigens specific to cancer cells is the foundation of the dendritic mRNA vaccine. These DCs, when appropriately activated, cause antigen-specific T lymphocytes to proliferate and develop into effector cells that can detect and destroy tumor cells regardless of their location.[15]

DCs must deliver tumor antigens to T cells in order to teach them to detect and destroy tumor cells in order to generate T-cell-mediated anti-tumor immunity.

Direct injection of non-replicating mRNA vaccines

Directly injected, non-replicating mRNA vaccines are intriguing vaccination formats due to their ease of administration and low cost, especially in resource-constrained situations.[16]

### 5.2 mRNA vaccine and covid-19

COVID-19 is caused by SARS-CoV-2, which is an enveloped virus with a positive-strand and single-stranded RNA genome that belongs to the β-coronavirus subfamily.[17]The advantages of mRNA in the new crown vaccine are mainly reflected in speed and safety. First, since the core of the development of mRNA vaccines lies in the sequence of antigens, once the nucleic acid of the virus is detected, the speed of development of mRNA vaccines will be much greater than that of traditional inactivated vaccines. The second is that compared with DNA vaccines, mRNA vaccines do not interfere with the host cell's genome (does not need to be transcribed in the nucleus), but are carried out directly in the cytoplasm. Furthermore, Protein-based Vaccines are usually produced by bacteria, while mRNA vaccines are translated by the host. Therefore, the translation mechanism may form an antigen, mimicking the structural genome of the protein expressed by the virus, including post-translational revision. It is remarkable to witness that two mRNA vaccines,mRNA-1273 and BNT162b were developed and manufactured in less than one year.[18] But there are also disadvantages. In addition to the stability that has been criticized (mRNA degradation), mRNA vaccines have extremely stringent requirements for transportation and storage conditions, which increase costs in disguise.

## 6. Conclusion

Research on mRNA vaccines should focus on increasing the stability of stable mRNA

Vaccines increase antigen reactivity, generate effective immunity, and ensure an effective delivery system.Covid-19 allowed us to see the infinite possibilities of mRNA vaccines, and greatly improved the level of vaccine development. In addition to the current problems that mRNA needs to solve, we should also apply mRNA to a wider range of fields, such as the prevention and treatment of microbial diseases. At the same time, we also hope that the development of mRNA vaccines will end this epidemic as soon as possible.

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