A Possible Blue Print of Addressing mRNA Vaccine Efficacy

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Abstract. As the COVID-19 pandemic progresses, more and more strategies to combat this pandemic have been proposed, and the mRNA vaccine against COVID-19 has been considered as one of the most potential therapies to terminate the pandemic. However, a few problems of it have been discovered during the whole investigation, such as instability, poor efficacy. Meanwhile, some researchers illustrated that improving the dose of mRNA vaccine to get better efficacy may induce worse adverse reactions due to the delivery of vaccine was 'lipid nanoparticles (LNPs)'. High level of LNPs would induce cell death by their toxicity, Therefore, some new modifications of mRNA should be paid attention and applied to produce the new mRNA vaccine. It is possible to promote the development of mRNA vaccine for better stability, efficacy, and lower price. This review introduces current modifications of mRNA vaccines for improved stability and discusses some novel ideas.

Keywords: mRNA Vaccine, Efficacy, Stability, Chemical Modification.

1. Introduction

Since November 9, 2022, COVID-19 has become one of the most widespread infectious diseases in human history, with 607,066,973 cases, as well as killed over 6.5 million people according to official statistics [1], while a paper published on Nature by David Adam demonstrated that the true death number caused by COVID-19 pandemic is more than 18 million by 31 December 2021 [2]. Therefore, some effective preventive or therapeutic methods to solve these serious problems have been a popular topic all around the world. The vaccine, especially mRNA vaccine, has been considered gradually as a potential approach to against COVID-19 pandemic.

Before the pandemic of COVID-19 happened, only few mRNA vaccine candidates were in early-stage clinical trials. The situation, however, of these had changed dramatically due to the efficiency, safety, high immunogenicity of mRNA vaccine, and it has been considered as an effective 'weapon' to against COVID-19 despite the challenges of developing mRNA vaccines. Now, Moderna, one of the most famous companies of mRNA vaccine, has published 10 clinical trials on its website, and there are 11 trials under the attitude of studying complete or recruitment complete [3]. Pfizer-BioNTech, as Moderna's contender, COVID-19 Vaccine is FDA authorized under Emergency Use Authorization (EUA) for active immunization to prevent COVID-19 pandemic [4]. Both of them have had considerable achievements on mRNA vaccine, which also brought them appreciable profits. Besides, not only the pandemic of COVID-19 needs the mRNA vaccine, but also other diseases, such as tumor, infectious diseases, need it as well. Researchers have claimed that the world need do somethings from the aspects of health-care systems, medicines, public-health officials to prepare for the future pandemic [5,6]. Hence, mRNA vaccine would occupy a necessary status in diverse vaccine candidates and how to improve the efficacy of mRNA vaccine has become a concerned topic in developing mRNA vaccine candidates.

2. Modification of mRNA vaccine impact on vaccine efficacy

Some studies have illustrated several methods to improve mRNA vaccinal delivery systems [7,8] and stability [9] for better efficacy, while a few researchers take more care of using delivery systems to increase mRNA vaccine efficacy rather than stability. The better stability of vaccine is necessary for a better mRNA vaccine, the reason behind that is better stability of vaccine will improve the condition of storage and the duration and efficiency of translation when the vaccine injected in the body and delivered into the cells thus increasing the vaccine efficacy effectively. Meanwhile, tuning

the stability of mRNA vaccine would cost less than optimizing better delivery systems, mRNA vaccine would also get less titer with more immunogenicity consequently and extend the expiration date so that it may lead the production of mRNA vaccines to reduce their price.

The Curevac, one of the most famous vaccinal companies, has failed in investigation of mRNA vaccine candidates because their vaccine only has 48% efficacy to against COVID-19 of any severity across all age groups and 15 variants [10]. Some scientists demonstrate that the dose of Curevac's mRNA vaccines, only 12 g vaccine each dose [10], is lower than other mRNA vaccines from Pfizer-BioNTech and Moderna, each dose has 30 g or 100 g vaccine [11,20]. However, the scientists of Curevac have done some experiments to find out the most suitable dose of mRNA vaccine, and they illustrated that from 2 g to 20 g, 12 g vaccine each dose is the best [10]. At the same time, Curevac's COVID-19 mRNA vaccines surrounded by lipid delivery, although the carrier has few differences, it is still generally same as the lipid delivery which Pfizer-BioNTech used. So, other researchers debated that the reason behind of lower efficacy is caused by chemical modification on mRNA vaccine.

In fact, all three companies' mRNA vaccines encode a coronavirus spike protein. But the Pfizer-BioNTech and Moderna mRNA vaccines use a nucleotide called pseudo uridine, a chemical modification occurs in uridine and change it chemical struct, in place of uridine itself [12]. By using this special uridine, it will avoid the reaction of inflammation in vivo which due to exogenous mRNA, and improve the stability of mRNA vaccine storage, but CureVac's mRNA vaccine used natural nucleotide. Innate immune system can identify unmodified mRNA, and then these mRNA will induce the reaction of immune to clear the exogenous substances [13] so that mRNA vaccine without chemical modification would be ineffective in body. As a result, Rein Verbeke, a scientist focuses on mRNA vaccinal investigate, demonstrate that modified mRNA had won this game [12]. So, although other functions of modification on mRNA vaccines are unknown, using modified nucleotide to replace the natural bases is imperative.

3. Current modifications of mRNA vaccines for improved stability

The natural structure of mRNA composed with a 5' and 3' untranslated region (UTR), and an open reading frame (ORF) region which encoding the protein, and there is a 7-methlguanosine cap structure (5'-cap, m7G) on the end of 5' UTR, also the 3' end has a ploy(A) tail structure. The component of mRNA vaccine is similar to the common mRNA, researchers pick up the 5' and 3' UTR from different genes which have longer half-life and are more stability, and then they compose these regions together to both ends of the mRNA vaccine candidates, in this way, due to these structures, the stability of mRNA vaccine will be enhanced, at the same time, the efficacy and translated efficiency of mRNA vaccine will also be improved [14,15].

The 5'-cap structure, as a chemical modification on the end of 5' UTR, can prevent mRNA degenerated by exonucleases so that maintains the translation and stability of mRNA vaccine [16]. According to the degree of methylation, the 5'-cap structure has been divided into three main categories: cap 0, cap 1, cap 2 [16]. Scientists mainly use cap 1 structure to modify the mRNA vaccine [17,18]. In Moderna, researchers applied the vaccinia caping enzyme (VCE) for making a cap 0 structure on the end of 5' UTR, and then they used 2'-O-MTase to shift the cap 0 to cap 1 by methylation, thereby achieved 100% efficiency for capping, and the production of COVID-19 mRNA vaccine mRNA-1273 has applied VCE for capping [17]. In Pfizer-BioNTech, researchers adopted the method, called CleanCap®, of capping mRNA during transcription of mRNA to achieve the co-transcriptional capping and finally capping a cap 1 structure on the end of 5' UTR [18]. At present, the production of COVID-19 mRNA vaccines BNT162b1 and BNT162b2 has used this capping technology to cap mRNA for cap 1 structure [19]. In conclusion, the cap structure not only improves mRNA vaccine stability, but also promotes the identification of host due to chemical modification thus improving vaccinal efficacy and the quality of vaccine [20].

The chemical modified nucleosides are core of the modified mRNA vaccine. Researchers have discovered some natural modified nucleosides on mRNA which would promote it stability and translational efficiency (Figure 1), and the host's innate immune system can identify unmodified mRNA as exogenous Mrna [21], thereby the immune system can interrupt the translation of mRNA *in vivo* [22]. Hence, researchers add modified nucleosides to mRNA vaccine, in place of natural bases, with certain proportion, in this way, they could improve the stability and translation efficiency of mRNA vaccine. At present, there is a method to modify the mRNA vaccine, the one is N1-methylpseudouridine (m1Ψ) which is always used to replace all the uridine nucleosides for better immunogenicity and stability [11,23]. However, the most natural modified nucleosides have never been used on mRNA vaccine, although some scientists found that using 25% of 5-methylcytidine (m5C) and 2-thiouridine (s2U) to replace the cytidine and uridine of mRNA will increase the translation efficiency and stability of mRNA in mice [24], the safety and the quality of vaccine is unknown. Hence, to improve the efficacy of 'second-generation' mRNA vaccine, the researchers should add other modified nucleosides except m1Ψ in place of natural bases.

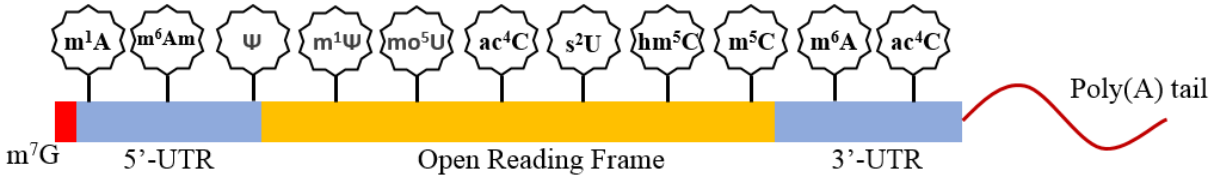

Figure 1. Natural modified nucleosides on mRNA [21]

4. Other modifications of mRNA would improve the stability of mRNA vaccine

Recently, mRNA modification has been a hot topic. The researchers have found a few modifications of mRNA since a few decades, these modifications also have diverse functions in regulating the expression of genes (Table 1). Besides m1Ψ, m5C, s2U has been investigated about replacing natural bases in mRNA ORF change it translation, immunogenicity, some new modification in 5' and 3' UTR has been found that it can also improve mRNA stability and translation efficiency. Meanwhile, 5' and 3' UTR has been considered as the region of regulating expression of genes, thereby some modification in these regions will only impact on mRNA stability rather than changing translation of mRNA, like N6-methyladenosine (m6A) and N4-acetylcytidine (ac4C), when both of them appear in mRNA, they would change the targeted mRNA translation efficiency, stability, alternative splicing, nuclear export [25,26]. However, they can only affect the stability of mRNA while they are modified in 3' UTR [26,28]. Hence, these modifications would be safer than other chemical modification which in ORF. Meanwhile, the researchers would not only use m1Ψ to replace the natural nucleosides, but also apply some new modified bases which in UTR to investigate a co-modified mRNA vaccine candidate. Therefore, scientists should develop new technology for producing other modified nucleosides and using these to replace normal bases by certain percentage in order to develop new-generation mRNA vaccine for more safety, efficient and stability.

Table 1. The functions of every mRNA modification

Modified type	Function	Reference
m^1A	Regulating the translation of protein	[27]
m^6Am	RNA stability	[26]
Ψ	RNA secondary structure, reducing immunogenicity of mRNA, improving translation efficiency	[12,15,23]
$m^1Ψ$	Reducing immunogenicity of mRNA, improving translation efficiency	[15,23]
mo^5U	Reducing immunogenicity of mRNA, improving translation efficiency	[15]
ac^4C	RNA stability, translation efficiency	[25]
s^2U	RNA stability, translation efficiency	[15,24]
hm^5C	Translation efficiency	[15,24]
m^5C	mRNA nuclear export	[15,24]
m^6A	RNA stability, translation, alternative splicing, nuclear export	[26]

5. Conclusions

COVID-19 pandemic has demonstrated that the health-system of every country and the reaction of pandemic still has a few leaks for against serious infectious diseases, and the economy of world is also facing serious challenges. Therefore, the researchers should find out some new methods for quickly responding to sudden pandemic. As these results, researchers with some biological corporations illustrate that mRNA vaccine would be considered as a best way to against the next pandemic rapidly due to the efficiency, safety, easy developing of it.

Although some mRNA vaccines have been permitted by government to produce or carried out clinical tails, the researcher would investigate 'next generation' mRNA vaccine candidates for better efficacy by optimizing the progression of mRNA vaccine production. Meanwhile, some people said that the next pandemic would occur in future 20 years with 50% possibility, thereby a new mRNA vaccine with better efficacy and quicker development is necessary.

At present, some researchers investigate that they developed some new delivered methods of mRNA vaccine due to the toxicity of LNPs which used to surround the mRNA currently. However, developing the LNPs or other delivery systems need a few nano-materials so that the progression of investigating these would spend much more money and time. Therefore, improving the mRNA vaccinal efficacy and stability by altering mRNA itself directly would be a preferred choice, and some researchers have gotten a few achievements about this by applying a chemical modification of m1Ψ to improve the immunogenicity and efficacy of mRNA vaccine. Hence, other modification of mRNA, like m6A, ac4C, m1A, m5C, et al, would be applied to develop next-generation mRNA vaccine candidates, although it would face to certain challenges. In this way, the world would prepare enough for next pandemic.

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