Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/44/20240857

mRNA therapeutics in diseases: Light inspect into a new horizon

Yanhui Li

Vanke School Pudong, Shanghai, 200000, China

yanhui.lee.0511@gmail.com

Abstract. An in-depth analysis of messenger RNA (mRNA) therapeutics and their potential to treat a wide range of diseases is provided in this thorough review paper. mRNA therapy has a great deal of potential for treating various terminal illnesses. By introducing mRNA as a vaccine or therapeutic agent, recent developments in biotechnology and molecular medicine have made it possible to produce almost any functional protein or peptide in the human body. This offers a unique chance to prevent and treat various challenging illnesses, many of which have genetic roots. Moreover, the utilization of in vitro transcribed mRNA has brought about a revolution in the production process, rendering it more efficient, adaptable, customizable, and cost-effective when compared to traditional methods. For a long time, researchers have aspired to enhance various aspects of mRNA, including its stability, immunogenicity, translation efficiency, and delivery systems, with the aim of achieving an efficient and secure means of delivering mRNA. In this comprehensive review, we will delve into the essence of mRNA and mRNA-based therapies, encompassing their fundamental principles, methods of production, diverse applications, outcomes, and constraints. We will underscore the importance of optimizing mRNA and its delivery systems to ensure the success of mRNA therapy, all while addressing the pivotal challenges and prospects in the battle against cancer and COVID-19.

Keywords: mRNA, LNP, Covid-19, Cancer, mRNA Therapeutics.

1. Introduction

1.1. The Essence of mRNA Function

At the nucleus of cellular function, mRNA emerges as a pivotal messenger, facilitating the transfer of genetic information from the DNA stored within a cell's nucleus to the machinery responsible for protein synthesis in the cytoplasm [1,2]. This process, known as transcription, hinges on creating single-stranded mRNA molecules that utilize DNA as a template. The mRNA sequence, deciphered as a sequence of three-base codons, acts as the blueprint guiding the assembly of amino acids into functional proteins [2]. This foundational process underpins the very essence of life as we comprehend it. However, the journey of mRNA into the host cytoplasm is fraught with formidable challenges. Its substantial size and negative charge present formidable barriers, impeding its passage through the cell membrane primarily composed of anionic lipids. Additionally, the innate immune system's capacity to engulf or degrade mRNA poses substantial obstacles, rendering the therapeutic use of mRNA a formidable challenge [2,3].

DOI: 10.54254/2753-8818/44/20240857

1.2. Pioneering Solutions: Innovative Delivery Mechanisms

To unlock the therapeutic potential of mRNA, visionary scientists have devised ingenious delivery systems. These encompass Lipid Nanoparticles (LNPs), Peptide-based delivery constructs, Polymer-based delivery platforms, Virus-like Replicon Particles (VRP), and Cationic Nanoemulsions (CNEs) [4]. Among these pioneering mechanisms, LNPs and polymer-based deliveries have emerged as particularly promising approaches, making significant strides in the treatment of a multitude of human diseases collectively referred to as mRNA therapeutics [4].

2. mRNA Therapeutics: A Brave New Frontier in Medicine

2.1. Transforming the Landscape of Disease Treatment

mRNA therapeutics represent a cutting-edge paradigm poised to redefine the treatment landscape for a diverse spectrum of diseases. When harnessed in conjunction with genetic engineering, synthetic mRNA empowers the expression of precise proteins within the patient's own body. The structural resemblance shared by synthetic mRNA with its natural counterpart allows patients to internally generate therapeutic proteins, circumventing the intricate challenges associated with the complex production of recombinant proteins [4]. This inherent versatility positions mRNA-based therapies as potent tools for treating diseases, enabling groundbreaking advancements in immunotherapies for cancer, pioneering stem cell therapies, and mastering infectious diseases [5]. In the context of the ongoing global COVID-19 pandemic, mRNA technology has unequivocally demonstrated its profound advantages, exemplified by the rapid development of vaccines that have heralded a new era in vaccine innovation.

2.2. Pioneering Beyond Protein Therapy: Gene Editing Horizons

Beyond protein therapies, the potential of mRNA extends into the realm of gene editing. Contemporary gene-editing techniques, such as *CRISPR-Cas9*, have grappled with formidable challenges, including the specter of off-target effects [6-8]. Here, mRNA emerges as a promising platform for gene-editing technologies. Its inherently short expression cycle reduces the presence of nucleases, substantially minimizing the risk of off-target effects. To sum up, RNA and mRNA play essential roles as the key conductors in the complex orchestration of life, facilitating the smooth transmission of genetic information from DNA to proteins. While the delivery of mRNA into cells presents formidable obstacles, innovative solutions have arisen, opening the gateway to mRNA therapeutics. These groundbreaking therapies possess the potential to reshape the contours of medicine, offering novel pathways for the treatment of diseases, the augmentation of immunity, and the advancement of gene editing techniques. The remarkable strides achieved in mRNA-based technology, exemplified by the rapid development of COVID-19 vaccines, underscore the transformative power of these molecules within the realm of biomedicine.

3. mRNA Therapeutics: A introduction to the vaccines

3.1. Understanding mRNA Vaccines

3.1.1. Classification of Vaccines. Vaccines are broadly categorized into two types: genotype-based and protein-based. Messenger RNA (mRNA) vaccines fall under the genotype-based category [4,5]. The fundamental principle of vaccine application involves delivering the vaccine to host cells using DNA or RNA as vectors. Once delivered, these vectors are expressed, stimulating an immune response from the host [7-9].

3.1.2. Advantages of mRNA Vaccines. mRNA vaccines, as a subtype of genotype-based vaccines, offer several advantages. Firstly, they are inherently safer. mRNA triggers a potent and enduring adaptive immune response by releasing cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-

DOI: 10.54254/2753-8818/44/20240857

alpha (IFN-α) from immune cells [9,10]. In contrast, traditional vaccines often require additional adjuvants to achieve this effect.

Secondly, mRNA, upon entry into cells, imposes minimal burdens. It remains confined to the cytoplasm without entering the cell nucleus. Additionally, mRNA exhibits a brief lifespan, easily degrading through natural physiological processes without lingering within the host's body. Moreover, the risk of rejection with mRNA vaccines is minimal due to their structural differences from DNA, lack of CpG islands, and distinct genomic integration.

Thirdly, mRNA vaccines are more cost-effective and straightforward to produce. They streamline the vaccine development process by satisfying all genetic information requirements for protein production through mRNA sequence modifications. This efficiency not only expedites vaccine development but also standardizes production processes across various mRNA vaccines, reducing time and costs.

3.2. Structure of mRNA Vaccines

The cap structure is an indispensable feature of mRNA vaccines. It consists of an N7-methylated guanosine at the 5' end of all mRNA molecules. This cap structure plays multiple roles, protecting mRNA from rapid degradation and facilitating the activation of eukaryotic initiation factors (eIFs) [11,12]. Three types of cap structures exist, Cap 0, Cap 1, and Cap 2, each serving distinct functions. Cap 0 recruits eIFs and shields mRNA from degradation while also stimulating interferon (IFN)-mediated responses. Additionally, the untranslated regions (UTRs) situated at the 5' and 3' termini of mRNA molecules assume crucial roles in cellular proteomics and the efficiency of translation. They impact the recruitment of ribosomes to mRNA and the choice of initiation codons [13,14].

3.3. Types of mRNA Vaccines

mRNA vaccines can be classified into three categories based on their genetic characteristics: non-replicating mRNA, self-amplifying RNA (saRNA), and circular RNA (CircRNA). Non-replicating mRNA serves to translate genetic information encoding the target antigen. It consists of an open reading frame (ORF), a 5' UTR containing the 7-methylguanosine cap structure (5'-cap, m7G), and a 3' UTR with a poly(A) tail structure, enhancing mRNA stability and translation accuracy [8,13].

In contrast, saRNA vaccines include an additional ORF compared to non-replicating mRNA. SaRNA inserts the gene encoding the target antigen into the RNA virus genome, allowing the antigen gene to self-amplify through viral replication mechanisms [15,16].

CircRNA, distinct from the other two vaccine types, possesses a covalent closed-loop structure. While non-replicating mRNA and saRNA are linear, CircRNA lacks cap-related translation elements but can be translated by incorporating IRES elements or m6A modifications into its 5' UTR. The closed-loop structure enhances CircRNA's stability, protecting it from exonuclease degradation [17].

It's noteworthy that non-replicating mRNA vaccines are the dominant players among COVID-19 vaccines in clinical trials or already available, whereas saRNA vaccines are undergoing preclinical and clinical development at various stages.

3.4. mRNA Delivery Systems

Due to their large size and negative charge, mRNA molecules cannot directly traverse the cell membrane. Additionally, they are susceptible to extracellular ribonuclease degradation. [18,19]. Therefore, mRNA delivery into cells for therapeutic purposes necessitates using transportation mediums [19,20]. Various mRNA delivery methods and vehicles have been developed and applied, leading to the classification of mRNA vaccines based on their delivery systems [21,22]. In conclusion, mRNA vaccines, a subset of genotype-based vaccines, offer unique advantages in terms of safety, minimal cellular impact, and cost-effective production. Their structure, comprising cap structures and UTRs, is critical in their functionality. mRNA vaccines come in different types, each with specific attributes, and their delivery systems are key to their therapeutic efficacy. Understanding these intricate details is paramount in harnessing the potential of mRNA vaccines in modern medicine [23,24].

DOI: 10.54254/2753-8818/44/20240857

4. Lipid Nanoparticles (LNPs): Advancements in mRNA Delivery

Due to the difficulties in natural delivery, some advanced pathways have been created. One of them is the Lipid Nanoparticles (LNPs), which are widely used in vaccine production [25,26]. In the domain of genetic engineering, the effective delivery of therapeutic mRNA molecules into cells has posed a formidable challenge. A groundbreaking solution has emerged in the form of LNPs. reshaping the landscape of RNA delivery [27-29]. LNPs, which consist of nanoscale particles composed of lipid materials or their derivatives, offer a multitude of advantages for mRNA-based therapies. This essay delves into the exceptional attributes of LNPs, underscoring their pivotal role in enabling efficient and secure mRNA delivery [25,30].

4.1. LNPs: The Adaptable mRNA Delivery Platform

LNPs possess a unique capability to encapsulate mRNA molecules within their lipid-based structure. This encapsulation serves as a protective shield, safeguarding the mRNA from enzymatic degradation and bolstering its stability during the delivery process [31,32]. The composition of LNPs can be meticulously customized to optimize their biophysical properties, ensuring efficient uptake by target cells. By employing a combination of synthetic or physiological lipid materials, LNPs are precisely engineered to form stable complexes with mRNA, creating a protective envelope that facilitates the successful transport of genetic material to its designated cellular destinations. [25,31]

4.2. Augmented Cellular Uptake and Intracellular Delivery

LNPs exhibit a remarkable advantage in their ability to facilitate the efficient uptake by cells and subsequent intracellular delivery of mRNA [33,34]. Following administration, cells recognize and internalize LNPs through endocytosis. These cellular mechanisms actively engulf LNPs, enabling the transfer of mRNA cargo into the cytoplasm. Within this cellular domain, mRNA can undergo efficient translation into therapeutic proteins. This targeted delivery strategy ensures that the intended genetic material reaches its precise site of action, optimizing its therapeutic efficacy [35,36].

4.3. Protection against Enzymatic Degradation

Within the harsh biological milieu, mRNA is highly susceptible to enzymatic degradation by nucleases. LNPs function as guardians, forming a protective shield around the encapsulated mRNA to shield it from enzymatic assaults [25,37]. The lipid-based shell establishes a safeguarding barrier that allows the mRNA payload to remain intact, significantly extending its half-life. This protection not only facilitates efficient delivery but also preserves the integrity of the therapeutic cargo, elevating the likelihood of successful protein expression within the target cells [33,38].

4.4. High Encapsulation Efficiency and Scalable Manufacturing

LNPs exhibit an impressive encapsulation efficiency, enabling the efficient packaging of a substantial quantity of mRNA within their nanoscale structure. This characteristic is particularly advantageous from a manufacturing perspective, as it enables the cost-effective production of large quantities of mRNA-loaded LNPs. The scalability of LNP production opens the door to broader access to mRNA-based therapies, ensuring that their potential benefits can be extended on a broader scale [18,39].

4.5. Safety Considerations

LNPs have demonstrated a favorable safety profile in both preclinical and clinical studies. The careful selection of lipid materials, combined with meticulous design and formulation, allows for the development LNPs with reduced toxicity and immunogenicity [37,40,41]. While some immune responses may occur due to the presence of the LNP carrier, these responses are generally mild and transient. Ongoing research and refinement of LNP formulations continue to strive for the optimization of safety profiles while minimizing potential adverse effects. In essence, Lipid Nanoparticles (LNPs) have emerged as a game-changing solution in the realm of mRNA delivery, offering an array of advantages that enhance the efficiency and safety of mRNA-based therapies. LNPs' adaptability, cellular

DOI: 10.54254/2753-8818/44/20240857

uptake, and protective capabilities make them indispensable in the advancement of genetic medicine and the realization of their therapeutic potential [42,43].

5. Utilizing mRNA Vaccines in the Cancer field

In the context of combating cancer, mRNA vaccines represent a promising avenue of research and application. These vaccines employ synthetic mRNA enclosed within protective structures that shield the mRNA from ribonucleases (RNases) and aid in effectively delivering target cells, tissues, and organs [44,45]. Upon entry, the mRNA activates Pattern Recognition Receptors (PRRs), initiating signal transduction cascades that lead to cytokine secretion and the development of adaptive immunity. This stands in contrast to other protein-based therapeutic approaches like enzyme replacement therapies (ERTs), which aim to restore enzyme activity [46-48].

Once within the cell's cytoplasm, mRNA vaccines initiate the transcription of therapeutic proteins in a host-cell-specific manner, offering a versatile approach to therapy [2]. mRNA vaccines also circumvent the challenges associated with delivering intracellular and transmembrane proteins encountered in ERTs,

Distinguishing themselves from DNA-based therapies, mRNA vaccines possess two key advantages: they pose no risk of genomic integration, as mRNA is translated in the cytoplasm without the need to enter the nucleus, and they do not require nuclear targeting. Additionally, the transient nature of mRNA means there is no risk of long-term contamination or residue [23,49].

Moreover, mRNA therapeutics are distinguished by their rapid, straightforward, and economical manufacturing process, primarily involving modifications to the RNA sequence to change the encoded protein. At the same time, the physical and chemical properties remain relatively constant [50-52]. This flexibility allows different products to be produced using the same production platform with minimal adjustments, saving both time and costs [53].

However, despite these advantages, effectively delivering mRNA to the ribosomes in the cytoplasm of target cells remains a significant challenge for clinical translation [8]. After endocytosis, mRNA often accumulates in lysosomes, leading to degradation, with only a small fraction reaching the cytoplasm. [54-56]. Additionally, mRNA, like other nucleic acids, is inherently unstable and susceptible to extracellular ribonuclease (RNase) degradation prior to internalization. Overcoming these hurdles is essential for developing highly efficient mRNA vaccines [49,57]. Due to progress in nanoscience, numerous nano-carriers have been developed to improve the delivery of mRNA into the cytoplasm. Among these non-viral vectors, lipid nanoparticles (LNPs) have gained substantial recognition and are extensively employed as vehicles for mRNA delivery [27,33,58].

LNPs represent intelligent, nano-sized lipid-based carriers that transport mRNA into the cell cytosol. Beyond safeguarding mRNA from RNases during systemic circulation, these granular nano-carriers efficiently deliver mRNA by fusing with lipid bilayers in early endosomes, facilitating the transport of mRNA to the cytoplasm [59-62]. Furthermore, research has shown that LNP formulations possess intrinsic adjuvant properties, promoting the generation of robust T follicular helper cells, germinal center B cells, long-lived plasma cells, and memory B cell responses, all of which contribute to the production of durable and protective antibodies in experimental models [63-65].

The composition of LNPs typically includes cationic ionizable lipids and helper lipids such as polyethylene glycol (PEG), phospholipids, and cholesterol. PEG lipids contribute to colloidal stability, preventing protein binding to nanoparticles, thereby reducing clearance by the reticuloendothelial system (RES) and extending systemic circulation. Phospholipids, usually neutral in nature (e.g., DSPC and DPPC), offer structural stability to LNPs in a bilayer configuration. Cholesterol, another neutral lipid component, enhances bilayer stability by increasing rigidity and preventing the leakage of therapeutic ingredients [30,39,66].

The mechanism underlying LNP-mediated mRNA delivery involves the formation of a complex between cationic ionizable lipids and mRNA, with this complex subsequently fusing with the endosomal membrane upon internalization, allowing mRNA release into the cytoplasm [26,59].

DOI: 10.54254/2753-8818/44/20240857

Despite the considerable progress made in LNP formulation technology that has enabled the development of mRNA vaccines, challenges persist, including the requirement for sub-zero storage and shipment temperatures and the potential for immune activation suppression induced by LNP-mRNA formulations.

It is important to note that mRNA vaccines, including their lipid nanoparticle (LNP) carriers and in vitro transcription (IVT) mRNA components, can elicit immune reactions that could affect the efficacy of the vaccine. Thus, in addition to various immunostimulatory byproducts or contaminants, such as double-stranded RNA (dsRNA), the desired mRNA is also produced [67,68].

Decreased translation, RNA degradation, and even apoptosis in target cells may result from an excessive immune response when the IVT reaction is not optimized and purity is compromised [69,70].

Furthermore, LNPs themselves can induce immune activation, as research has shown various in vivo immune effects associated with them, including immune cell activation, inflammation, and adaptive immune responses [71-73]. While LNP-based mRNA vaccines have become a reality in developed countries, there is a need for further research to better understand the structure and characteristics of LNPs. Additionally, improving methods such as enhancing their storage stability is crucial to expand their applications, especially in the field of cancer research [74,75].

The potential of mRNA vaccines in the context of cancer can be exemplified through an experiment conducted by Cafri G and colleagues based on the widely accepted theory that neoantigen-specific T cells are present in most cancers [76,77]. In this experiment, metastatic tumors are harvested, and tumor-infiltrating lymphocytes (TILs) are cultured for future testing. Each resected tumor and its corresponding peripheral blood mononuclear cell (PBMC) samples are sequenced to identify tumor-specific mutations. Subsequently, high-throughput immunological screening is employed using long peptides and tandem minigenes (TMGs) covering all mutated epitopes to isolate purified antigen sequences [78,79]. With these identified antigens as a foundation, mRNA vaccines can be developed to elicit specific T-cell immune responses, enhancing the efficient clearance of malignant cells. This approach holds great promise for advancing cancer immunotherapy [80,81].

6. mRNA vaccines and the Covid-19

In the process of in vitro transcription (IVT) for mRNA synthesis, several components come into play, including a cap, ribonucleotides, a DNA template featuring a promoter, and a phage RNA polymerase that recognizes this promoter [82]. As a consequence of this process, the desired mRNA is generated alongside various immunostimulatory byproducts or contaminants like double-stranded RNA (dsRNA) [83]. Consequently, when the IVT reaction is not optimized, and purity is compromised, it can trigger an exaggerated immune response, potentially leading to diminished translation, RNA degradation, and even apoptosis in target cells [84].

Among the various COVID-19 vaccine candidates in development, two have shown exceptional promise in preventing COVID-19 infection and represent a novel class of vaccines [85]. These vaccines utilize messenger ribonucleic acid (mRNA) strands encapsulated in lipid nanoparticles (LNPs).[74]. Upon intramuscular injection, LNPs facilitate the uptake of the mRNA into host cells, where the mRNA sequence is translated into the spike protein (S protein) [22,25]. Following post-translational modifications within the host cell, the S protein is displayed on the cell surface as a membrane-bound antigen in a pre-fusion conformation, providing an antigenic target for B cells [86,87]. Additionally, a portion of the newly synthesized spike protein enters the antigen presentation pathway, presenting T cell epitopes via major histocompatibility complexes (MHC) to activate T cells [88,89].

This mRNA-based approach offers several advantages for combating COVID-19. The production of mRNA vaccines is a rapid and straightforward process. Once the mRNA sequence encoding the desired immunogen is established, RNA synthesis can commence immediately on the same platform, allowing for swift and adaptable production of various mRNA vaccines [23,24]. mRNA vaccines offer a greater degree of biological safety when compared to DNA-based vaccines. This enhanced safety is a result of mRNA translation taking place in the cytoplasm, reducing the risk of genomic integration that can occur

DOI: 10.54254/2753-8818/44/20240857

with DNA-based vaccines, which necessitate entry into the nucleus. Additionally, the shorter sequence of mRNA further diminishes the chances of interactions with the host genome [4,59,90].

In clinical trials, some temporary side effects have been observed, including pain, soreness, fatigue, headache, muscle pain, and fever, although these reactions are typically short-lived. Other adverse events, such as chills, joint pain, nausea, itching, rash, itchy throat, and mild respiratory symptoms, have been reported less frequently [91,92]. Interestingly, phase III trial results from Moderna have demonstrated that the vaccine maintains over 90% efficacy against the virus even six months after the second dose. Similarly, an experimental trial involving 307 participants revealed that the Pfizer vaccine maintained 91.3% efficacy six months after the second dose. However, it's crucial to approach vaccine efficacy based on phase III clinical trial results with caution [93,94].

To optimize the pharmacological aspects of mRNA, several technologies are currently being employed. Synthetic cap analogues and capping enzymes have been shown to stabilize mRNA and enhance protein translation. The 5' cap construct, in particular, prevents mRNA degradation by exonucleases, ensuring mRNA stability and facilitating translation initiation. Regulatory elements within the 5'-untranslated region (UTR) and the 3'-UTR of mRNA have been found effective in stabilizing mRNA and enhancing protein translation. The poly(A) tail also plays a crucial role in maintaining mRNA stability and translation efficiency [14,95].

Additionally, nucleoside modifications have been explored to enhance mRNA stability and translational capacity while reducing its immunogenicity in vivo. Techniques like high-performance liquid chromatography (HPLC) and fast protein liquid chromatography (FPLC) purification have been used to decrease immune activation and increase mRNA translation efficiency [96,97]. Collectively, these strategies aim to enhance the efficacy and safety of mRNA-based vaccines, making them a promising tool in the fight against COVID-19 and potentially other diseases.

7. Conclusion

The advent of mRNA vaccines, coupled with the groundbreaking LNP transmission technology, marks the dawn of a new era in mRNA therapeutics. The transformative impact of these advancements has been vividly demonstrated in the realms of both cancer treatment and the recent battle against COVID-19. As we delve deeper into the implications of this innovative approach, it becomes apparent that the potential of mRNA therapeutics extends far beyond the boundaries we have explored so far.

The multifaceted applications of mRNA technology offer a promising avenue for developing novel treatments across various diseases. Beyond the immediate successes witnessed in cancer and infectious disease management, the ongoing exploration of mRNA's potential in diverse therapeutic areas unveils a vast landscape of possibilities. The versatility of mRNA as a therapeutic tool opens doors to previously uncharted territories, inviting further investigation and discussion.

In essence, the journey into the realm of mRNA therapeutics is only just beginning. The profound impact witnessed in cancer and COVID-19 represents just the tip of the iceberg, and the full spectrum of possibilities is yet to be unveiled. As we stand on the cusp of this new horizon, the continuous exploration and refinement of mRNA technologies promise a future where the boundaries of disease treatment are pushed beyond conventional limits. The unfolding chapters in the mRNA story present an exciting narrative that invites researchers, clinicians, and the scientific community at large to delve into the vast potential that mRNA therapeutics holds for shaping the future of medicine.

References

[1] Jian Y. (2023), Ji Yu Xin Shi He Tang He Suan (mRNA) Zhi Liao - Guo Qu, Xian Zai He Wei Lai: [The therapeutic base on mRNA - past, now and future]. Medical Research & Publication. https://doi.org/10.14218/mrp.2023.004

[2] Wolff, J. A., Malone, R. W., Williams, P., Chong, W., Acsadi, G., Jani, A., & Felgner, P. L. (1990). Direct gene transfer into mouse muscle in vivo. Science (New York, N.Y.), 247(4949 Pt 1), 1465–1468. https://doi.org/10.1126/science.1690918

[3] Xu, S., Yang, K., Li, R., & Zhang, L. (2020). mRNA Vaccine Era-Mechanisms, Drug Platform and Clinical Prospection. International journal of molecular sciences, 21(18), 6582. https://doi.org/10.3390/ijms21186582
[4] Qin S, Tang X, Chen Y, et al. mRNA-based therapeutics: powerful and versatile tools to combat diseases. Signal Transduction and Targeted Therapy 2022; 7(1), https://doi.org/10.1038/s41392-022-01007-w
[5] Delivery of mRNA therapeutics. European Pharmaceutical Review January 6, 2023. https://www.europeanpharmaceuticalreview.com/article/177409/mrna-therapeutics/#:~:text=mRNA%20technologies%20have%20emerged%20as,diseases%2C%20as%20well%20as%20cancer.
[6] Linares-Fernández, S., Lacroix, C., Exposito, J. Y., & Verrier, B. (2020). Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends in molecular medicine, 26(3), 311–323. https://doi.org/10.1016/j.molmed.2019.10.002
[7] Sahin, U., Karikó, K., & Türeci, Ö. (2014). mRNA-based therapeutics--developing a new class of drugs. Nature reviews. Drug discovery, 13(10), 759–780. https://doi.org/10.1038/nrd4278
[8] Kowalski, P. S., Rudra, A., Miao, L., & Anderson, D. G. (2019). Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. Molecular therapy : the journal of the American Society of Gene Therapy, 27(4), 710–728. https://doi.org/10.1016/j.ymthe.2019.02.012
[9] Park, J. W., Lagniton, P. N. P., Liu, Y., & Xu, R. H. (2021). mRNA vaccines for COVID-19: what, why and how. International journal of biological sciences, 17(6), 1446–1460. https://doi.org/10.7150/ijbs.59233
[10] Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines - a new era in vaccinology. Nature reviews. Drug discovery, 17(4), 261–279. https://doi.org/10.1038/nrd.2017.243
[11] Ramanathan A, Robb GB, Chan S-H. mRNA capping: biological functions and applications. Nucleic Acids Research 2016; 44(16):7511–7526. doi:10.1093/nar/gkw551.
[12] Cowling VH. Regulation of mRNA cap methylation. Biochemical Journal 2009; 425(2):295–302. doi:10.1042/bj20091352.
[13] Perenkov AD, Sergeeva AP, Vedunova MV, Krysko DV. In vitro transcribed RNA-Based platform vaccines: past, present, and future. Vaccines 2023; 11(10):1600. doi:10.3390/vaccines11101600.
[14] Kim SC, Sekhon SS, Shin W, et al. Modifications of mRNA vaccine structural elements for improving mRNA stability and translation efficiency. Molecular & Cellular Toxicology 2021; 18(1):1–8. doi:10.1007/s13273-021-00171-4.
[15] Zhou W, Jiang L, Liao S, et al. Vaccines' New Era-RNA vaccine. Viruses 2023; 15(8):1760. doi:10.3390/v15081760.
[16] Fang E, Liu X, Li M, et al. Advances in COVID-19 mRNA vaccine development. Signal Transduction and Targeted Therapy 2022; 7(1). doi:10.1038/s41392-022-00950-y.
[17] Qu L, Yi Z, Shen Y, et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. Cell 2022; 185(10):1728-1744.e16. doi:10.1016/j.cell.2022.03.044.
[18] Liu T, Tian Y, Zheng A, Cui C. Design strategies for and stability of mRNA–Lipid Nanoparticle COVID-19 vaccines. Polymers 2022; 14(19):4195. doi:10.3390/polym14194195.
[19] Nitika, Wei J, Hui A. The delivery of mRNA vaccines for therapeutics. Life 2022; 12(8):1254. doi:10.3390/life12081254.
[20] Zhang W, Jiang Y, He Y, et al. Lipid carriers for mRNA delivery. Acta Pharmaceutica Sinica B 2023; 13(10):4105–4126. doi:10.1016/j.apsb.2022.11.026.
[21] Xu S, Yang K, Li R, Zhang L. MRNA Vaccine Era—Mechanisms, drug platform and Clinical Prospection. International Journal of Molecular Sciences 2020; 21(18):6582. doi:10.3390/ijms21186582.

[22] Zeng C, Zhang C, Walker P, Dong Y. Formulation and delivery technologies for mRNA vaccines. In: Current Topics in Microbiology and Immunology; 2020:71–110.
[23] Liu T, Liang Y, Huang L. Development and delivery systems of mRNA vaccines. Frontiers in Bioengineering and Biotechnology 2021; 9. doi:10.3389/fbioe.2021.718753.
[24] Schlake T, Theß A, Fotin-Mleczek M, Kallen KJ. Developing mRNA-vaccine technologies. RNA Biology 2012; 9(11):1319–1330. doi:10.4161/rna.22269.
[25] Wilson B, Geetha K. Lipid nanoparticles in the development of mRNA vaccines for COVID-19. Journal of Drug Delivery Science and Technology 2022; 74:103553. doi:10.1016/j.jddst.2022.103553.
[26] Swetha KL, Kotla NG, Tunki L, et al. Recent advances in the lipid Nanoparticle-Mediated delivery of mRNA vaccines. Vaccines 2023; 11(3):658. doi:10.3390/vaccines11030658.
[27] Jung HN, Lee S, Lee S, Youn H, Im H. Lipid nanoparticles for delivery of RNA therapeutics: Current status and the role of in vivo imaging. Theranostics 2022; 12(17):7509–7531. doi:10.7150/thno.77259.
[28] Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nature Reviews Materials 2021; 6(12):1078–1094. doi:10.1038/s41578-021-00358-0.
[29] Hınçer A, Ahan RE, Aras E, Şeker UÖŞ. Making the next generation of therapeutics: mRNA meets synthetic biology. ACS Synthetic Biology 2023; 12(9):2505–2515. doi:10.1021/acssynbio.3c00253.
[30] Albertsen CH, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Advanced Drug Delivery Reviews 2022; 188:114416. doi:10.1016/j.addr.2022.114416.
[31] Guimarães PPG, Zhang R, Spektor R, et al. Ionizable lipid nanoparticles encapsulating barcoded mRNA for accelerated in vivo delivery screening. Journal of Controlled Release 2019; 316:404–417. doi:10.1016/j.jconrel.2019.10.028.
[32] Guimaraes, P. P. G., Zhang, R., Spektor, R., Tan, M., Chung, A., Billingsley, M. M., El-Mayta, R., Riley, R. S., Wang, L., Wilson, J. M., & Mitchell, M. J. (2019). Ionizable lipid nanoparticles encapsulating barcoded mRNA for accelerated in vivo delivery screening. Journal of controlled release : official journal of the Controlled Release Society, 316, 404–417. https://doi.org/10.1016/j.jconrel.2019.10.028
[33] Aldosari BN, Alfagih IM, Almurshedi AS. Lipid nanoparticles as delivery systems for RNA-Based vaccines. Pharmaceutics 2021; 13(2):206. doi:10.3390/pharmaceutics13020206.
[34] Kim S, Choi B, Kim Y, Shim G. Immune-Modulating lipid nanomaterials for the delivery of biopharmaceuticals. Pharmaceutics 2023; 15(6):1760. doi:10.3390/pharmaceutics15061760.
[35] Golubovic A, Tsai S, Li B. Bioinspired lipid nanocarriers for RNA delivery. ACS Bio & Med Chem Au 2023; 3(2):114–136. doi:10.1021/acsbiomedchemau.2c00073.
[36] Pozzi D, Caracciolo G. Looking back, moving forward: lipid nanoparticles as a promising frontier in gene delivery. ACS Pharmacology & Translational Science 2023. doi:10.1021/acsptsci.3c00185.
[37] Yang L, Gong L, Wang P, et al. Recent advances in lipid nanoparticles for delivery of mRNA. Pharmaceutics 2022; 14(12):2682. doi:10.3390/pharmaceutics14122682.
[38] Schoenmaker L, Witzigmann D, Kulkarni JA, et al. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. International Journal of Pharmaceutics 2021; 601:120586. doi:10.1016/j.ijpharm.2021.120586.
[39] Tenchov R, Bird RE, Curtze A, Zhou Q. Lipid Nanoparticles─From liposomes to mRNA Vaccine delivery, a landscape of research diversity and advancement. ACS Nano 2021; 15(11):16982–17015. doi:10.1021/acsnano.1c04996.
[40] Mehta M, Bui TA, Yang X, Aksoy YA, Goldys EM, Deng W. Lipid-Based Nanoparticles for Drug/Gene Delivery: An Overview of the production techniques and difficulties encountered in their industrial development. ACS Materials Science Au 2023; 3(6):600–619. doi:10.1021/acsmaterialsau.3c00032.

[41] Mashima R, Takada S. Lipid Nanoparticles: a novel gene delivery technique for clinical application. Current Issues in Molecular Biology 2022; 44(10):5013–5027. doi:10.3390/cimb44100341.
[42] Ndeupen S, Qin Z, Jacobsen S, Bouteau A, Estanbouli H, Igyártó BZ. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. iScience 2021; 24(12):103479. doi:10.1016/j.isci.2021.103479.
[43] Korzun T, Moses AS, Diba P, et al. From bench to bedside: Implications of lipid nanoparticle carrier reactogenicity for advancing nucleic acid therapeutics. Pharmaceuticals 2023; 16(8):1088. doi:10.3390/ph16081088.
[44] Chehelgerdi M, Chehelgerdi M. The use of RNA-based treatments in the field of cancer immunotherapy. Molecular Cancer 2023; 22(1). doi:10.1186/s12943-023-01807-w.
[45] Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. Therapeutic Delivery 2016; 7(5):319–334. doi:10.4155/tde-2016-0006.
[46] Mandolfo O, Parker H, Bigger B. Innate immunity in mucopolysaccharide diseases. International Journal of Molecular Sciences 2022; 23(4):1999. doi:10.3390/ijms23041999.
[47] Jh J, Shin HW, Lee JM, Lee H, Kim E-C, Park SH. An overview of pathogen recognition receptors for innate immunity in dental pulp. Mediators of Inflammation 2015; 2015:1–12. doi:10.1155/2015/794143.
[48] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010; 140(6):805–820. doi:10.1016/j.cell.2010.01.022.
[49] Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and challenges in the delivery of mRNA-Based vaccines. Pharmaceutics 2020; 12(2):102. doi:10.3390/pharmaceutics12020102.
[50] Liu A, Wang X. The pivotal role of chemical modifications in mRNA therapeutics. Frontiers in Cell and Developmental Biology 2022; 10. doi:10.3389/fcell.2022.901510.
[51] Webb C, Ip S, Bathula NV, et al. Current status and future perspectives on MRNA drug manufacturing. Molecular Pharmaceutics 2022; 19(4):1047–1058. doi:10.1021/acs.molpharmaceut.2c00010.
[52] Qin S, Tang X, Chen Y, et al. mRNA-based therapeutics: powerful and versatile tools to combat diseases. Signal Transduction and Targeted Therapy 2022; 7(1). doi:10.1038/s41392-022-01007-w.
[53] Javaid M, Haleem A, Singh RP, Suman R. Enabling flexible manufacturing system (FMS) through the applications of industry 4.0 technologies. Internet of Things and Cyber-Physical Systems 2022; 2:49–62. doi:10.1016/j.iotcps.2022.05.005.
[54] Qin S, Tang X, Chen Y, et al. mRNA-based therapeutics: powerful and versatile tools to combat diseases. Signal Transduction and Targeted Therapy 2022; 7(1). doi:10.1038/s41392-022-01007-w.
[55] Li M, Li Y, Li S, et al. The nano delivery systems and applications of mRNA. European Journal of Medicinal Chemistry 2022; 227:113910. doi:10.1016/j.ejmech.2021.113910.\
[56] Li D, Liu Q, Yang M, et al. Nanomaterials for mRNA-based therapeutics: Challenges and opportunities. Bioengineering & Translational Medicine 2023; 8(3). doi:10.1002/btm2.10492.
[57] Pardi N, Hogan MJ, Porter F, Weissman D. mRNA vaccines — a new era in vaccinology. Nature Reviews Drug Discovery 2018; 17(4):261–279. doi:10.1038/nrd.2017.243.
[58] Zhang W, Jiang Y, He Y, et al. Lipid carriers for mRNA delivery. Acta Pharmaceutica Sinica B 2023; 13(10):4105–4126. doi:10.1016/j.apsb.2022.11.026.
[59] Ramachandran S, Satapathy SR, Dutta T. Delivery strategies for mRNA vaccines. Pharmaceutical Medicine 2022; 36(1):11–20. doi:10.1007/s40290-021-00417-5.
[60] Tenchov R, Bird RE, Curtze A, Zhou Q. Lipid Nanoparticles─From liposomes to mRNA Vaccine delivery, a landscape of research diversity and advancement. ACS Nano 2021; 15(11):16982–17015. doi:10.1021/acsnano.1c04996.

[61] Tilstra G, Couture-Senécal J, Lau YMA, et al. Iterative design of ionizable lipids for intramuscular mRNA delivery. Journal of the American Chemical Society 2023; 145(4):2294–2304. doi:10.1021/jacs.2c10670.
[62] Qin S, Tang X, Chen Y, et al. mRNA-based therapeutics: powerful and versatile tools to combat diseases. Signal Transduction and Targeted Therapy 2022; 7(1). doi:10.1038/s41392-022-01007-w.
[63] Alameh M-G, Tombácz I, Bettini E, et al. Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. Immunity 2021; 54(12):2877-2892.e7. doi:10.1016/j.immuni.2021.11.001.
[64] Han X, Alameh M-G, Butowska K, et al. Adjuvant lipidoid-substituted lipid nanoparticles augment the immunogenicity of SARS-CoV-2 mRNA vaccines. Nature Nanotechnology 2023; 18(9):1105–1114. doi:10.1038/s41565-023-01404-4.
[65] Chi X, Gu J, Thomas R. Characteristics and roles of T follicular helper cells in SARS-COV-2 vaccine response. Vaccines 2022; 10(10):1623. doi:10.3390/vaccines10101623.
[66] Chan C, Du S, Dong Y, Cheng X. Computational and experimental approaches to investigate lipid nanoparticles as drug and gene delivery systems. Current Topics in Medicinal Chemistry 2021; 21(2):92–114. doi:10.2174/1568026620666201126162945.
[67] Pilkington EH, Suys EJA, Trevaskis NL, et al. From influenza to COVID-19: Lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. Acta Biomaterialia 2021; 131:16–40. doi:10.1016/j.actbio.2021.06.023.
[68] Kwon S, Kwon M, Im S, Lee K, Lee H. mRNA vaccines: the most recent clinical applications of synthetic mRNA. Archives of Pharmacal Research 2022; 45(4):245–262. doi:10.1007/s12272-022-01381-7.
[69] Thomas M, Liu X, Whangbo J, et al. Apoptosis Triggers Specific, Rapid, and Global mRNA Decay with 3′ Uridylated Intermediates Degraded by DIS3L2. Cell Reports 2015; 11(7):1079–1089. doi:10.1016/j.celrep.2015.04.026.
[70] Ouranidis A, Vavilis T, Mandala E, et al. mRNA Therapeutic Modalities Design, Formulation and Manufacturing under Pharma 4.0 Principles. Biomedicines 2021; 10(1):50. doi:10.3390/biomedicines10010050.
[71] Lee Y, Jeong M, Park J, Jung H, Lee H. Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. Experimental & Molecular Medicine 2023; 55(10):2085–2096. doi:10.1038/s12276-023-01086-x.
[72] Igyártó BZ, Jacobsen S, Ndeupen S. Future considerations for the mRNA-lipid nanoparticle vaccine platform. Current Opinion in Virology 2021; 48:65–72. doi:10.1016/j.coviro.2021.03.008.
[73] Qin Z, Igyártó BZ. Pre-exposure to mRNA-LNP inhibits adaptive immune responses and alters innate immune fitness in an inheritable fashion. bioRxiv (Cold Spring Harbor Laboratory) 2022. doi:10.1101/2022.03.16.484616.
[74] Schoenmaker L, Witzigmann D, Kulkarni JA, et al. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. International Journal of Pharmaceutics 2021; 601:120586. doi:10.1016/j.ijpharm.2021.120586.
[75] Fayez NA, Nassar MS, Alshehri AA, et al. Recent advancement in mRNA vaccine development and applications. Pharmaceutics 2023; 15(7):1972. doi:10.3390/pharmaceutics15071972.
[76] Ni L. Advances in mRNA-Based cancer vaccines. Vaccines 2023; 11(10):1599. doi:10.3390/vaccines11101599.
[77] Cafri G, Gartner JJ, Zaks T, et al. mRNA vaccine–induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. Journal of Clinical Investigation 2020; 130(11):5976–5988. doi:10.1172/jci134915.
[78] Creelan BC, Wang C, Teer JK, et al. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. Nature Medicine 2021; 27(8):1410–1418. doi:10.1038/s41591-021-01462-y.

[79] Hall M, Teer JK, Yu X, et al. Neoantigen-specific CD4+ tumor-infiltrating lymphocytes are potent effectors identified within adoptive cell therapy products for metastatic melanoma patients. PubMed 2023; 11(10). doi:10.1136/jitc-2023-007288.
[80] Li Y, Wang M, Peng X, et al. mRNA vaccine in cancer therapy: Current advance and future outlook. Clinical and Translational Medicine 2023; 13(8). doi:10.1002/ctm2.1384.
[81] Kaczmarek M, Poznańska J, Fechner F, et al. Cancer Vaccine Therapeutics: Limitations and Effectiveness—A Literature Review. Cells 2023; 12(17):2159. doi:10.3390/cells12172159.
[82] Perenkov AD, Sergeeva AP, Vedunova MV, Krysko DV. In vitro transcribed RNA-Based platform vaccines: past, present, and future. Vaccines 2023; 11(10):1600. doi:10.3390/vaccines11101600.
[83] Youssef M, Hitti C, Fulber JPC, Kamen A. Enabling mRNA Therapeutics: Current landscape and challenges in manufacturing. Biomolecules 2023; 13(10):1497. doi:10.3390/biom13101497.
[84] Mu X, Hur S. Immunogenicity of In Vitro-Transcribed RNA. Accounts of Chemical Research 2021; 54(21):4012–4023. doi:10.1021/acs.accounts.1c00521.
[85] Li Y, Tenchov R, Smoot JW, Liu C, Watkins SC, Zhou Q. A comprehensive review of the global efforts on COVID-19 vaccine development. ACS Central Science 2021; 7(4):512–533. doi:10.1021/acscentsci.1c00120.
[86] Fung TS, Liu DX. Post-translational modifications of coronavirus proteins: roles and function. Future Virology 2018; 13(6):405–430. doi:10.2217/fvl-2018-0008.
[87] Xia X. Domains and functions of spike protein in SARS-COV-2 in the context of vaccine design. Viruses 2021; 13(1):109. doi:10.3390/v13010109.
[88] Rijkers GT, Weterings N, Obregón-Henao A, et al. Antigen presentation of mRNA-Based and Virus-Vectored SARS-COV-2 vaccines. Vaccines 2021; 9(8):848. doi:10.3390/vaccines9080848.
[89] Giannotta G, Giannotta N. MRNA COVID-19 vaccines and Long-Lived Plasma Cells: a complicated relationship. Vaccines 2021; 9(12):1503. doi:10.3390/vaccines9121503.
[90] Park J, Lagniton PNP, Liu Y, Xu RH. mRNA vaccines for COVID-19: what, why and how. International Journal of Biological Sciences 2021; 17(6):1446–1460. doi:10.7150/ijbs.59233.
[91] Dhamanti I, Suwantika AA, Adlia A, Yamani LN, Yakub F. Adverse reactions of COVID-19 vaccines: A scoping review of observational studies. International Journal of General Medicine 2023; Volume 16:609–618. doi:10.2147/ijgm.s400458.
[92] Guo Y, Tian X, Wang X, Xiao Z. Adverse effects of immunoglobulin therapy. Frontiers in Immunology 2018; 9. doi:10.3389/fimmu.2018.01299.
[93] Patel R, Kaki M, Potluri VS, Kahar P, Khanna D. A comprehensive review of SARS-CoV-2 vaccines: Pfizer, Moderna & Johnson & Johnson. Human Vaccines & Immunotherapeutics 2022; 18(1). doi:10.1080/21645515.2021.2002083.
[94] Fiolet T, Kherabi Y, MacDonald C, Ghosn J, Peiffer-Smadja N. Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. Clinical Microbiology and Infection 2022; 28(2):202–221. doi:10.1016/j.cmi.2021.10.005.
[95] Warmiński M, Mamot A, Depaix A, Kowalska J, Jemielity J. Chemical modifications of mRNA ends for therapeutic applications. Accounts of Chemical Research 2023; 56(20):2814–2826. doi:10.1021/acs.accounts.3c00442.
[96] Zhang J, Liu Y, Li C, et al. Recent Advances and Innovations in the Preparation and Purification of In Vitro-Transcribed-mRNA-Based Molecules. Pharmaceutics 2023; 15(9):2182. doi:10.3390/pharmaceutics15092182.
[97] Koppu V, Poloju D, Puvvala B, et al. Current Perspectives and Future Prospects of mRNA Vaccines against Viral Diseases: A Brief Review. PubMed 2022; 11(3):260–272. doi:10.22088/ijmcm.bums.11.3.260.