Research Progress of Mrna Therapy: From Concept to Clinic

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Abstract. mRNA therapeutics have emerged as a highly promising approach with the potential to revolutionize disease treatment. This review is to provide a comprehensive exploration of the fundamental principles and practical applications of mRNA therapy, from its conceptualization to clinical experimentation. Through a thorough examination of literature and analysis of clinical trial data, this review highlights the pharmaceutical potential of mRNA, including its adaptable design and efficient production. The advancements made in addressing challenges such as mRNA degradation and immunogenicity through modifications, purification techniques, and delivery systems are also discussed. Furthermore, it provides an overview of the progress in clinical trials of mRNA therapeutics, showcasing the promising developments in this field. Overall, this review emphasizes the significant contributions and implications of mRNA therapeutics in disease treatment, underscoring its potential to revolutionize the field and hold great promise for the future.

Keywords: mRNA, mRNA Vaccine, Clinical Trial, Lipid Nanoparticle.

1. Introduction

Messenger RNA (mRNA) plays a pivotal role as an indispensable intermediate in the process of protein synthesis, comprising a single-stranded chain of ribonucleotides. Owing to their diverse array of advantages, a remarkable surge in the development of mRNA-based vaccines has been witnessed during the outbreak of the COVID-19 pandemic.

At present, mRNA therapy, which is a medical approach that uses synthetic messenger RNA molecules to instruct cells to produce specific proteins, offering a promising treatment option for various diseases, has found extensive applications across multiple domains, including vaccines targeting the novel coronavirus [1], influenza virus [2], and even tumor treatments [3-4]. Many of these applications have successfully transitioned into clinical experimentation. However, despite the exponential growth of research in this field in recent years, a comprehensive review encompassing the progress of mRNA-based therapies remains conspicuously absent.

Therefore, this review aims to demonstrate the discovery and basic knowledge of mRNA, comprehensively survey the advantages and challenges encountered in the formulation of mRNA-based therapeutics and proffer viable resolutions. Meanwhile, the review also aims to introduce commonly employed carrier systems in mRNA therapy and culminate in a systematic collation of ongoing clinical trials predicated on mRNA technology.

2. Discovery and pharmaceutical development of mRNA

2.1. Discovery of mRNA

During the early stages of scientific exploration, Francis Crick et al. engaged in speculations regarding a potential correlation between proteins and DNA, putting forth the notion of "template RNA", later recognized as ribosomes [5]. However, the precise involvement of mRNA in the intricate process of protein synthesis remained shrouded in uncertainty. It was not until the late 1950s and early 1960s that Arthur Pardee [6], Jacques Monod [7], Francois Jacob [8], and Matthew Meselson successfully identified mRNA as the crucial messenger molecule responsible for conveying instructions from the nucleus to ribosomes. They not only confirmed the pivotal role of mRNA in bacterial systems but also established its indispensable centrality in the intricate process of protein

synthesis. Subsequently, the experiments conducted by Marshall Nirenberg and Heinrich Matthaei [9] in the late 1960s provided compelling evidence of mRNA's direct and active participation in the complex process of protein synthesis, which effectively decrypted the genetic code and further substantiated the notion that mRNA serves as the conduit through which genetic information is faithfully transcribed into functional proteins.

2.2. Exploration of mRNA pharmaceutical development

The exploration of the potential of mRNA drugs has been underway since 1978. Researchers have conducted experiments by directly injecting mRNA drugs into animals or using modified mRNA or carriers to improve drug stability and reduce immunogenicity. In 1987, Robert Malone had first succeeded in human cell in vitro mRNA transfection and accomplished transfecting mRNA in living organisms in the next year [10-11]. Since then, the medicinal and therapeutic potential of mRNA has been already put forward. In 2017, personalized cancer vaccines utilizing mRNA began their first human trials with the development of lipid nanoparticle technology. These vaccines aimed to enhance T-cell response against cancer by targeting specific mutant epitopes. The results showed increased immune activity and reduced disease recurrence in high-risk patients, indicating the promising application of mRNA as a medicine, particularly in the field of cancer treatment [12]. Now mRNA-based therapies have a wide range of applications in the fight against cancer, infectious diseases, allergies, protein replacement therapy, and so on. Nevertheless, it was not until 2019, after the outbreak of COVID-19 and the approval of the first COVID mRNA vaccine BNT162b2 by the FDA that led to the widespread recognition of the capacity of mRNA-based therapeutic agents [13].

3. Advantage and challenges of mRNA therapy

3.1. Advantage of mRNA therapies

Compared to DNA therapies (Table 1), one core advantage of mRNA therapies arises from its role as an endogenous intermediate template in protein synthesis, enabling mRNA to efficiently direct protein synthesis without the risk of genomic integration. Additionally, mRNA does not require nuclear localization to mediate protein translation, simplifying the intracellular protein expression process [14].

Compared to conventional vaccines (Table 1), mRNA therapies involve the administration of an order that directs the synthesis of the target structure instead of injecting the medicine or antigen as a product. This allows for a longer duration since both the material and the workplaces are contained within the body, constituting a second advantage of mRNA therapy. Research findings indicate that mRNA vaccines, including the Pfizer/BioNTech and Moderna vaccines, demonstrate superior antibody response in terms of both peak levels and long-lasting protection when compared to natural infections and the two vector vaccines, namely the AstraZeneca and Johnson & Johnson vaccines [15]. In addition, mRNA medicines do not contain any core components of the virus. In contrast, the conventional inactivated virus vaccine involves injecting the complete virus structure into the human body, which carries a certain risk of toxicity. mRNA, on the other hand, accurately generates the antigenic structure of the virus through protein translation without producing toxic side effects. This minimizes the potential harm from other structures of the virus and reduces the risk of accidental infection.

mRNA drugs offer a shorter development cycle and higher production capacity since there is no need for virus-specific steps such as virus inactivation in the production process [16]. In contrast, conventional vaccines often require re-development based on virus characteristics. mRNA vaccines can be quickly and flexibly adapted by adjusting the alignment of the base sequences, offering a significant advantage in controlling rapidly evolving viruses like COVID-19. In practical production, a single product line can be used to produce therapies for various diseases, facilitating the mass production of vaccines against outbreak viruses and rapid variations.

mRNA also exhibits a high immune efficacy, which is achieved through dual immunization involving humoral and T cells. Experimental data provide evidence of the biological significance of cross-reactive memory CD4+ T cells [17]. Additionally, mRNA vaccines are highly immunogenic, eliminating the need for adjunctive vaccine adjuvants and thereby reducing the potential side effects associated with such substances.

Table.1. Common vaccine types and their advantages/disadvantages

	Category	Advantage	Disadvantage
Traditional Vaccines	Live-attenuated	Induces humoral and cellular immunity Strong and long-lasting immune response Diverse vaccination methods	Retained virulence: potential restoration Vacuum freeze-drying process: difficult storage and transport
	Inactiwated	Easy to store	Lower immunogenicity Multiple doses required Adjuvant addition needed
	Subunit	Removal of reactive components: improved safety and stability	Small size and low immunogenicity: requires conjugation with protein carrier
Novel Vaccines	DNA	Cell-free production Innate adjuvant effect Easy to produce and store Coding all the epitopes	Potential risk in mutation insertion Need to be transfected into nuclear
	mRNA	Cell-free production Transient activity Easy to be degraded Coding all the epitopes	Easy to be degraded Hard to store
	Protein/peptide	Cell-free production Transient activity and easy to be degraded	Hard to produce at clinical use level Potential side immune responses
	Antigen loaded DCs	Strong immune responses	High production cost

3.2. Challenges in mRNA pharmaceutical development

Despite the advantages mentioned, challenges for mRNA are still tricky on the road to becoming known as drugs. Firstly, RNA, in general, has a reputation for unbelievable instability [12], which poses a number of challenges for both their preservation and transportation. In contrast to DNA, the mRNA molecule has a linear single-stranded structure, and the 2' and 3' positions of the ribose residues contain a large number of hydroxyl groups, especially the 2' hydroxyl group, which is vulnerable to attack and hydrolysis. In addition, there are a lot of free RNases in the human body, which makes it easy to be degraded by extracellular endonuclease [13]. Thus, the main routes of mRNA degradation are physical and chemical degradation, both of which are primarily influenced by base sequence and mRNA secondary structure [16]. By the way, the degradation pattern of RNA molecules is consistent with the Arrhenius model [18].

Also, naked mRNA, being a foreign substance to the body, elicits an immune response. Therefore, the implementation of an appropriate delivery system, along with essential modifications and alterations, becomes imperative to render mRNA capable of safely entering the cell and successfully expressing the target proteins, rather than being eliminated by the body's immune system.

Moreover, mRNA itself has a high density of negative charge, which makes it difficult to directly penetrate the cell membrane into the cell, in addition, it has a high natural immunogenicity, which also causes low in vivo transmission efficiency, difficult to play an effective role [13].

mRNA holds immense promise in the field of pharmaceuticals owing to its numerous advantages. It offers flexibility in production, eliminating concerns associated with genomic integration observed in DNA therapeutics. Additionally, mRNA-based vaccines mitigate the risk of incomplete inactivation leading to infections, a concern associated with inactivated or attenuated vaccines. However, the inherent challenges of mRNA, namely its instability and high immunogenicity, necessitate overcoming these obstacles to fully exploit its potential. To enable the widespread application of mRNA vaccines in clinical settings, several targeted strategies have been devised and implemented.

4. mRNA modifications for mRNA therapy

4.1. Base substitution

Base substitution, specifically codon optimization, is a widely used method to enhance mRNA stability. This involves increasing the GC content as well as minimizing the presence of uridine. To address the issue of mRNA immunogenicity, pseudouridine has been identified as a suitable alternative to uridine [19]. mRNA containing pseudouridine has emerged as an ideal molecule for therapy, as it exhibits improved translation efficiency and does not induce interferon in mice, unlike unmodified mRNA. Uridine substitution in mRNA is also advantageous for tolerance induction, enhanced protein expression, and cost-efficient mRNA production. This substitution prevents excessive innate immune activation and facilitates type I interferon production. Furthermore, uridine modification allows for fine-tuning of the immune response and ensures efficient antigen expression without the risk of nuclear entry or genome integration [20].

4.2. Chemical modifications

Chemical modifications target specific regions within the mRNA structure to enhance translation efficiency, improve stability, simplify the production process, reduce immunogenicity, and achieve other objectives. Currently, methods such as the addition of caps [21] and tails, as well as modifications to UTR segments [22], are considered relatively mature. However, ongoing research is continuously exploring newer approaches in these areas.

Among the various processing methods of mRNA, the addition of the 5'-cap and poly(A) tail to the head and tail of mRNA, respectively, is an important one in mainstream applications. The 5'-cap not only prevents mRNA from being degraded by exonucleases but also interacts with specific enzymes and proteins, directing mRNA turnover and processing, including splicing, nuclear export, and translation initiation. Additionally, the 5'-cap protects mRNA from triggering a strong immune response, and its methylation status helps distinguish self from non-self RNA [21].

Based on the traditional method of adding a 5'-cap and poly(A) tail, an innovative mRNA has been developed that uses an internal ribosomal entry site (IRES) along with terminal hairpins as substitutes for the cap and tail. This substitution aims to protect the 5' and 3' ends of mRNA from exonucleases [23]. Besides, phosphorothioate cap analogs, a recently synthesized series of cap analogs with improved properties that contain a sulfur substitution for a nonbridging oxygen, contribute to higher translational efficiency and stability as well [24]. During the capping process of mRNA synthesis in vitro, effective cap analogs coincide with invalid anti-reverse cap analogs, which significantly reduce raw material utilization and productivity. To combat this issue, novel "anti-reverse" cap analogs with superior translational properties come out [25].

Other processing methods focus on the 5'- and 3'-UTR regions of mRNA. The UTR, or untranslated region, is a segment of several hundred nucleotides that precedes the mRNA translation initiation codon. It is also referred to as the leading sequence region. For instance, a high-throughput

strategy has been developed to designing, screening, and optimizing 5'-UTRs that enhance protein expression from a strong human cytomegalovirus (CMV) promoter [22].

4.3. Alternative optimization strategies

In addition to mRNA modification, other innovative approaches can be employed to enhance the stability, efficiency, and protein expression of mRNA. For example, dual chemical modifications have been found to synergistically improve these properties, attracting the attention of researchers. For example, a combination of natural and unnatural base modifications has been developed, utilizing the site-specificity of unnatural nucleotides in the 3'-UTR and the improved translation efficiency of natural base modifications and 5mC [26]. Another novel direction is the exploration of mRNA structure optimization, where highly structured "superfolder" mRNAs can be designed to enhance stability and expression, further enhanced by pseudouridine nucleoside modification [27]. Additionally, specific optimization algorithms can be investigated to improve stability and immunogenicity, complementing existing principles [28].

To achieve superior quality mRNA, purification to obtain impurity-free mRNA is also crucial in enhancing therapeutic efficacy. However, physical separation of uncapped and capped mRNA poses challenges due to their nearly identical physicochemical properties [29]. In this regard, hydrophobic photocaged tag-modified cap analogs [30] have been developed to facilitate the separation of capped mRNA from uncapped mRNA via reversed-phase high-performance liquid chromatography. This method significantly enhances the translational activity of mRNA by 3-4 times.

5. mRNA delivery systems

Besides the production and modification of mRNA molecules, delivering mRNA to target cells or tissues efficiently and effectively is a significant and inevitable problem during the development of mRNA-based therapeutic systems. As mentioned above, mRNA poses challenges due to its high negative charge density, making it challenging to penetrate the cell membrane and enter the cell directly. Additionally, its inherent immunogenicity further hampers its in vivo transmission efficiency, limiting its effectiveness [13]. Therefore, the development of safe and efficient mRNA delivery systems is a crucial and unavoidable problem for mRNA-based therapy technologies.

The development of nucleic acid delivery technology dates back to the 1970s [31]. After more than 50 years of development, nucleic acid delivery technology has been widely used in biological experiments. One of the more common ones is physical delivery: physical delivery methods, including electroporation, microinjection, laser-mediated transfection, and so on [32-33]. Physical nucleic acid transfection does not rely on specific carriers, but rather physically destroys the integrity of the membrane or alters the permeability of the membrane, allowing the exogenous nucleic acid to directly enter the cell, which tends to have the advantages of high efficiency, accuracy, and a wide range of applications. However, the effects of the instrumentation and experimental manipulation involved in these methods themselves on the state of the cells should not be ignored, and these factors will also reduce the stability of physical delivery methods to some extent [34-35].

However, non-physical methods of nucleic acid delivery are more attractive. Using existing technology, scientists have been able to achieve carrier-free delivery of nucleic acids by modifying the nucleic acid itself to avoid degradation by the immune system and enhance phagocytosis of target cells [36-37]. In addition, there are delivery methods based on viral, virus-like, or non-viral vectors, as well as polymers and metals. These carrier-based methods have shown great potential in the biomedical field due to their good stability, high in vivo delivery efficiency, extensive functional modification, and the possibility of mass-integrated production [38]. These features are of great interest. Many carrier-based nucleic acid delivery systems have been validated in clinical trials.

The construction of mRNA carrier complexes based on nanotechnology is a current priority selection to cater to the clinical needs of therapeutic mRNA delivery. Currently, various types of nanocarriers are being widely developed for the efficient delivery of mRNA. At present, various types

of nanocarriers are being widely developed for the effective delivery of mRNA. As mentioned above, it is expected that these vectors can effectively improve the stability of mRNA, its translation efficiency, achieve the targeting of tissues and cells, as well as optimize the manufacturing process and storage conditions of these vectors as much as possible. Among them, viral vectors have excellent performance in mRNA delivery and translation efficiency. However, their complex preparation process, limited loading capacity, together with their strong immunogenicity greatly reduce their flexibility as mRNA vectors [39]. Non-viral vectors, on the other hand, can be chemically tailored to be more versatile to meet different delivery needs. In the following, we will introduce several common mRNA non-viral vectors.

Lipid-based delivery systems are already a well-established class of mRNA delivery vectors that are widely known and have been used in a very wide range of clinical applications. More specifically, the vast majority of lipid-based mRNA delivery systems are nanoparticles with diameters between tens and hundreds of nm, which are generally called lipid nanoparticles (LNPs). In addition to the Lipid-based or LNP-based mRNA vaccines (BNT162b2, mRNA-1273) that we are already familiar with, this type of delivery systems are also widely used for the delivery of various drugs, including but not limited to anti-cancer drugs such as doxorubicin (Lipodox), Daunorubicin (DaunoXome), and Paclitaxel (Abraxane); various small nucleic acids: siRNAs (Onpattro, Inclisiran), miRNAs (MRX34), and other small nucleic acid-based drugs and others that are being used in a wide range of clinical applications [40-41].

6. Clinical studies of mRNA therapy

In order to investigate the clinical progress of mRNA therapy, we conducted a comprehensive search on the clinical trial website to identify all clinical trials in Phase II and beyond that utilized mRNA therapy as a treatment. Subsequently, we constructed a dedicated database comprising these trials. Analysis of these clinical trials revealed that a majority of them were in the early stages, with 72.5% classified as Phase I or Phase II trials. Notably, a significant proportion of the ongoing RNA therapy clinical trials, accounting for 61 out of 263 trials in our database (Table 2), were based on mRNA. These trials are being further optimized for different patient populations to obtain additional approvals. This phenomenon highlights the pivotal role played by the emergence of the COVID-19 pandemic in driving the development of mRNA vaccines and suggests that the application of mRNA vaccines in various other diseases can be considered as an extension or variant of COVID-19 vaccines.

Among the numerous clinical trials, the Phase I/II study of the COVID-19 RNA vaccine BNT162b1 in adults, registered under the identifier NCT04368728, serves as a representative experiment. The clinical trial conducted in the study involved evaluating the safety, tolerability, and immunogenicity of the COVID-19 RNA vaccine BNT162b1 in healthy adults aged 18-55 years. The participants were randomly assigned to receive either the vaccine or a placebo, with safety assessments including observation for adverse events, self-reporting of reactions, and laboratory assessments. The study found that the vaccine induced dose-dependent local reactions and systemic events, with increased RBD-binding IgG concentrations and SARS-CoV-2 neutralizing titres after the second dose. The results supported further evaluation of the mRNA vaccine candidate. Follow-up will continue for up to 2 years to assess long-term safety and immunogenicity. The study also highlighted the importance of including older adults and diverse populations in future phases of the research 107 [42].

Additionally, other notable clinical trials based on mRNA therapy have been compiled in Table 2.

Table.2. Clinical trials of mRNA therapy

NCT Number	Conditions	Phases	Enrollment	First Posted	Study URL
NCT05537571	Cardiovascular Diseases|Atherosclerosis|Lipoprotein(a)	PHASE2	160	2022/9/13	https://clinicaltrials.gov/study/NCT05537571
NCT04775069	Chronic Liver Disease	PHASE4	900	2021/3/1	https://clinicaltrials.gov/study/NCT04775069
NCT05672355	Chronic Lymphocytic Leukemia|COVID-19 Infection	PHASE2	80	2023/1/5	https://clinicaltrials.gov/study/NCT05672355
NCT05343871	COVID-19	PHASE4	2340	2022/4/25	https://clinicaltrials.gov/study/NCT05343871
NCT05924685	COVID-19 Vaccines	PHASE4	110	2023/6/29	https://clinicaltrials.gov/study/NCT05924685
NCT05212610	COVID-19|Corona Virus Infection	PHASE4	200	2022/1/28	https://clinicaltrials.gov/study/NCT05212610
NCT05085366	Cytomegalovirus Infection	PHASE3	7300	2021/10/20	https://clinicaltrials.gov/study/NCT05085366
NCT05169489	Diffuse Large B Cell Lymphoma (DLBCL)	PHASE1|PHASE2	50	2021/12/27	https://clinicaltrials.gov/study/NCT05169489
NCT05556720	HIV|Organ Transplantation|Lymphoma, Non-Hodgkin	PHASE3	960	2022/9/27	https://clinicaltrials.gov/study/NCT05556720
NCT05970887	Immune Response|Safety	PHASE4	154	2023/8/1	https://clinicaltrials.gov/study/NCT05970887
NCT05580159	Immunogenicity|Efficacy|Safety	PHASE3	2000	2022/10/14	https://clinicaltrials.gov/study/NCT05580159
NCT05911087	Immunogenicity|Safety	PHASE2|PHASE3	800	2023/6/20	https://clinicaltrials.gov/study/NCT05911087
NCT04844840	Keloid	PHASE2	60	2021/4/14	https://clinicaltrials.gov/study/NCT04844840
NCT05077254	Kidney Transplant Recipients|Liver Transplant Recipients	PHASE2	400	2021/10/14	https://clinicaltrials.gov/study/NCT05077254
NCT05975099	Lyme Disease	PHASE1|PHASE2	800	2023/8/3	https://clinicaltrials.gov/study/NCT05975099
NCT05028374	Multiple Myeloma|AL Amyloidosis	PHASE2	119	2021/8/31	https://clinicaltrials.gov/study/NCT05028374
NCT05028361	Pain|Quality of Life|Injection Site Reaction	PHASE4	349	2021/8/31	https://clinicaltrials.gov/study/NCT05028361
NCT05119855	Papillomavirus Infections|Coronavirus Disease (COVID-19)	PHASE3	160	2021/11/15	https://clinicaltrials.gov/study/NCT05119855
NCT05158140	Pneumococcal Infection	PHASE3	850	2021/12/15	https://clinicaltrials.gov/study/NCT05158140
NCT05499013	Polycythemia Vera	PHASE1|PHASE2	65	2022/8/12	https://clinicaltrials.gov/study/NCT05499013
NCT05330975	Respiratory Syncytial Virus	PHASE3	3354	2022/4/15	https://clinicaltrials.gov/study/NCT05330975
NCT05236491	Rheumatoid Arthritis|Autoimmune Rheumatologic Disease	PHASE2|PHASE3	287	2022/2/11	https://clinicaltrials.gov/study/NCT05236491
NCT04792567	Secondary Progressive Multiple Sclerosis	PHASE4	41	2021/3/11	https://clinicaltrials.gov/study/NCT04792567
NCT05822908	Spinocerebellar Ataxia Type 1|Spinocerebellar Ataxia Type 3	PHASE1|PHASE2	65	2023/4/21	https://clinicaltrials.gov/study/NCT05822908

6.1. COVID-19 mRNA vaccines

With the rapid development of the mRNA vaccine field catalyzed by the COVID-19 pandemic, various mRNA COVID-19 vaccine candidates have matured, with some already progressing into clinical trials and receiving regulatory approval. Among them, Pfizer-BioNTech vaccine (BNT162b2) [43], Moderna vaccine (mRNA-1273) [44] and CureVac vaccine (CVnCoV) [45] are the most renowned products that have been authorized for emergency use or approved for market launch. On December 11, 2020, the vaccine BNT162b2, jointly developed by Pfizer and BioNTech, received emergency use authorization from the FDA, becoming the first mRNA-based COVID-19 vaccine approved for human use [46]. Shortly after, the Moderna vaccine mRNA-1273 was also authorized for use in the United States. Ultimately, they were among the first batch of SARS-CoV-2 vaccines authorized in the United States, United Kingdom, Canada, and several other countries.

The successful development and global application of COVID-19 mRNA vaccines represent a milestone for mRNA therapy. Pfizer-BioNTech evaluated the BNT162b2 mRNA vaccine and found that it had an overall efficacy of 95% in preventing COVID-19, which greatly marked the efficacy of mRNA therapy. The vaccine was effective across different age groups, with efficacy rates of 95.6% in individuals aged 16 to 55 years, 93.7% in individuals over 55 years, and 94.7% in individuals over 65 years. The vaccine also showed high efficacy in different racial and ethnic groups, with rates ranging from 89.3% to 100%. The study included participants from different countries, including Argentina, Brazil, and the United States, and the vaccine demonstrated high efficacy in all these populations [43]. Clinical trial results indicate that vaccine recipients generate a robust immune response, leading to significant achievements in preventing COVID-19 infections and reducing disease transmission.

Apart from efficacy, longer duration can also be achieved. mRNA vaccines, such as mRNA-1273 and BNT162b2, offer more durable protection against breakthrough infections compared to natural infection, with a median duration of 29.6 months and 21.5 months, respectively 1. On the other hand, viral vector vaccines like ChAdOx1 and Ad26.COV2. S are projected to provide lower and shorter-term protection against breakthrough infections, with median durations of 22.4 months and 20.5 months, respectively [47].

Furthermore, the safety and immunogenicity of Sinovac-CoronaVac have been examined in 12-17-year-olds who had adverse reactions to the Pfizer-BNT162b2 mRNA vaccine. Results showed the boosters were safe, with no serious adverse events. T-cell responses were observed, particularly against the Spike protein, lasting up to 150 days. Neutralizing antibody levels with Sinovac-CoronaVac were comparable to Pfizer-BNT162b2, with variations based on COVID-19 infections [48].

As an emerging vaccine type, the post-approval deployment of mRNA vaccines has raised concerns regarding their safety, particularly in special populations such as the elderly, children and individuals with underlying medical conditions. Eimear Kelly et al. aimed to investigate the reactogenicity and immunogenicity of heterologous or fractional second dose COVID-19 vaccine regimens in adolescents. The researchers recruited 148 participants aged 12 to 16 years and randomized them to receive either 30 μg BNT162b2 (BNT-30), 10 μg BNT162b2 (BNT-10), or NVX-CoV2373 (NVX) as a second dose, 8 weeks after a first dose of 30 μg BNT162b2 in order to study solicited systemic reactions in the week following vaccination and included immunogenicity and safety. The study found that the heterologous and fractional dose COVID-19 vaccine schedules in adolescents were safe, well-tolerated, and immunogenic. The NVX-CoV2373 schedule showed enhanced performance against the Omicron SARS-CoV-2 variant, suggesting it may provide greater protection than the licensed homologous schedule [1].

6.2. Influenza vaccine

Researchers have explored the use of mRNA vaccines for influenza, offering a new era in vaccinology [2]. A clinical trial (NCT03076385) involving an mRNA vaccine for Influenza virus has enrolled 22 participants. mRNA vaccines have the potential to address challenges in vaccine

development for both infectious diseases and cancer. Kapil Bah et al. demonstrate that lipid nanoparticle-formulated mRNA vaccines encoding hemagglutinin proteins of H10N8 and H7N9 generated strong immune responses in mice, ferrets, and nonhuman primates. A single dose of the H7N9 mRNA vaccine protected mice from a lethal challenge and reduced lung viral titers in ferrets. Interim results from a phase 1 H10N8 study in humans showed high seroconversion rates and acceptable tolerability profiles. The authors conclude that mRNA vaccines can induce protective immunogenicity with minimal adverse events [49]. mRNA-based influenza vaccines have shown promising results in preclinical and early clinical trials.

6.3. Human Monkeypox (hMPX) vaccine

A human Mpox (hMPX) epidemic began in 2022, creating a huge and urgent demand for a monkeypox vaccine. In response, Heng Xia et al. developed a new vaccine and tested its immunogenicity and efficacy in mice. The vaccine is composed of mRNA-lipid nanoparticles (mRNA-LNPs) encoding four highly conserved Mpox virus surface proteins that are involved in the attachment, entry and transmission of the virus, namely A29L, A35R, B6R and M1R, which are homologous to the A27, A33, B5 and L1 of the vaccinia virus (VACV). These vaccines provide protection against VACV challenges, including reduced weight loss and death, by inducing Mpox virus-specific IgG antibodies and potent VACV-specific neutralizing antibodies. Experiments have demonstrated that injecting mice with 5 μg of A27, B5, and L1 mRNA-LNPs twice, or a low dose of averaging a mixture of these four antigenic mRNA-LNPs (0.5 μg each) twice, protects mice from weight loss and death after VACV challenge. In addition, serum protection experiments, and mouse challenge experiments were conducted to verify the efficacy and safety of the vaccine [50]. The findings suggest that these mRNA-LNP vaccine candidates are safe and effective against MPXV and other orthopoxviruses.

6.4. Cancer preventive vaccine

Cancer, as the second major application field of mRNA therapy, has also witnessed significant advancements in mRNA therapy. Clinical trials have been conducted using mRNA vaccines encoding neoantigens for various types of cancer, including advanced melanoma [3], lung cancer, colon cancer, pancreatic cancer, and solid tumors [4]. These trials have shown promising results, such as significant T-cell responses in advanced melanoma patients and the secretion of proinflammatory mediators and peripheral T-cell responses in patients with various cancers. Additionally, mRNA vaccines have been found to be safe and well-tolerated in patients with solid tumors. The use of mRNA vaccines in clinical settings demonstrates their potential as a novel approach to cancer immunotherapy, with ongoing trials and advancements showing promise for cancer treatment.

Ugur Sahin et al. investigates the efficacy of melanoma FixVac (BNT111), a liposomal RNA vaccine targeting non-mutated tumour-associated antigens in patients with advanced melanoma. Conducted in a phase I trial [51], the vaccine demonstrates promising outcomes in checkpoint-inhibitor (CPI)-experienced patients with unresectable melanoma. Treatment with melanoma FixVac, either alone or in combination with PD1 checkpoint inhibitor, leads to durable objective responses accompanied by robust CD4+ and CD8+ T cell immune responses against the vaccine antigens. Some responders exhibit potent and enduring antigen-specific cytotoxic T-cell resp onses akin to adoptive T-cell therapy levels. These findings underscore the potential of RNA vaccination as a potent immunotherapy for CPI-experienced melanoma patients and highlight the significance of non-mutant shared tumour antigens as viable targets for cancer vaccination [51].

Alexandros Papachristofilou et al. aims to evaluate the safety and efficacy of BI1361849, an mRNA-based cancer immunotherapy, combined with local radiation treatment in patients with stage IV non-small cell lung cancer (NSCLC). The results showed that the treatment was well tolerated, with injection site reactions and flu-like symptoms being the most common adverse events. Immunomonitoring revealed increased antigen-specific immune responses in the majority of patients, and some patients showed partial response or stable disease as the best overall response. The study

supports further investigation of mRNA-based immunotherapy in NSCLC, including combinations with immune checkpoint inhibitors [52].

7. Conclusion and outlook

mRNA therapeutics have garnered significant attention and are poised to revolutionize disease treatment. Since its discovery as an intermediate product in protein synthesis, mRNA has been recognized for its immense pharmaceutical potential. Researchers have made remarkable progress in addressing challenges associated with mRNA, such as degradation and immunogenicity, through modifications, purification techniques, and the development of delivery systems. These advancements have paved the way for the development of highly promising mRNA vaccines, which have demonstrated efficacy in various fields, including the prevention and treatment of diseases like COVID-19 and cancer. Notably, several mRNA therapeutics have already entered clinical trials, reflecting the rapid progress in this field driven by the demand created by the COVID-19 pandemic.

However, to overcome the inherent limitations of mRNA, including instability and high immunogenicity, the development of carrier systems with enhanced stability, improved transfection efficiency, increased functionality, and precise targeting capabilities is crucial for the transition of mRNA from a conceptual stage to large-scale clinical application. Currently, significant strides have been made in carrier research, with various types of foundational carriers demonstrating successful delivery of mRNA into the body and fulfilling their intended roles. Moving forward, research and development efforts will increasingly focus on multifunctional carriers and precise targeting, as these areas hold immense potential for facilitating the development of more effective and safer mRNA therapy products.

With approved treatments and a wide range of potential applications, mRNA therapeutics hold profound implications for addressing diverse diseases. The recent advancements and ongoing research in this field, accompanied by the demand created by the COVID-19 pandemic, point towards a momentous decade ahead. The future of mRNA therapeutics appears promising, as it offers a transformative approach to disease treatment and opens new avenues for personalized and effective therapies.

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