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REVIEW

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Emergence of mRNA vaccines in the management of cancer

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ABSTRACT

Introduction: mRNA vaccines have been developed as a promising cancer management. It is noted that specification of the antigen sequence of the target antigen is necessary for the design and manufacture of an mRNA vaccine.

Areas covered: The steps involved in preparing the mRNA-based cancer vaccines are isolation of the mRNA cancer from the target protein using the nucleic acid RNA-based vaccine, sequence construction to prepare the DNA template, *in vitro* transcription for protein translation from DNA into mRNA strand, 5′ cap addition and poly(A) tailing to stabilize and protect the mRNA from degradation and purification process to remove contaminants produced during preparation.

Expert opinion: Lipid nanoparticles, lipid/protamine/mRNA nanoparticles, and cell-penetrating peptides have been used to formulate mRNA vaccine and to ensure vaccine stability and delivery to the target site. Delivery of the vaccine to the target site will trigger adaptive and innate immune responses. Two predominant factors of the development of mRNA-based cancer vaccines are intrinsic influence and external influence. In addition, research relating to the dosage, route of administration, and cancer antigen types have been observed to positively impact the development of mRNA vaccine.

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Cancer; preventive; vaccination; vaccine delivery; vaccine preparation

1. Introduction

Based on statistics provided by WHO (2022), roughly 10 million deaths, or nearly one in six deaths, will be caused by cancer in 2020, making it the top cause of death globally. The most prevalent cancers are prostate, breast, lung, colon, and rectum. In 2020, it is stated that the most prevalent cancer is breast with 2.26 million cases, followed by lung with 2.21 million cases, colon and rectum with 1.93 million cases, prostate with 1.41 million cases, skin (non-melanoma) with 1.20 million cases, followed by stomach with 1.09 million cases [1].

Each type of cancer requires a different approach of therapy and a precise cancer diagnosis is crucial for proper and successful care. Surgery, radiation, systemic therapies, such as chemotherapy, hormonal medicines, and targeted biological therapies, are frequently used in treatment [2,3]. Additionally, in recent years, immunotherapy has emerged as a groundbreaking approach of cancer treatment. Miao et al. reported that mRNA vaccines have progressed into a promising cancer therapeutic platform [2]. As mRNA can serve as an efficient vector for the delivery of therapeutic antibodies on immunological targets, it is becoming increasingly popular in cancer immunotherapy. Immunotherapies for the treatment of cancer have been made possible by advancements in immune system knowledge. Different immune cells can recognize antigens on the surface of cancer cells, and they can then engage with the antigenic peptides to kill the cancer cells [4]. Antigen-presenting cells (APCs) are cells that help the innate and adaptive immune system function. During immunization, naked or vehicle-loaded mRNA vaccines effectively express tumor antigens in APCs [2].

Besides, the idea of implementing therapeutic vaccination to treat cancer has been developed by researchers for decades. The SARS-CoV-2 mRNA vaccines' quick development and global approval have demonstrated the technology's immense potential. The unprecedented success of mRNA vaccines against infection has proved its effectiveness. The development of mRNA-based cancer vaccines has been improved in response to the COVID-19 pandemic by analyzing data from years of research. Early clinical trial results only provided modest evidence of clinical efficacy. However, with the optimization of mRNA vaccine structure, stability, and administration methods, along with the related benefits of

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Article highlights

- There are two types of mRNA vaccine which are non-replicating mRNA and replicating mRNA. Non-replicating mRNA vaccine has the benefits of the transcript can be produced more economically and integrated into lipid nanoparticles more easily.
- Replicating mRNA has received a lot of attention due to its long-lasting effectiveness and low dosage requirement.
- Synthetic mRNA molecules used in the mRNA vaccines regulate the production of the antigen that will trigger an immunological response.
- Preparation of mRNA-based cancer vaccine includes isolation of mRNA vaccine, sequence construction, in vitro transcription, 5′ cap addition, poly(A) tail production, and purification of DNA template.
- Early cancer vaccination therapies relied on self-antigens known as tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs).
- RNA vaccine is superior to DNA vaccine; it only needs to enter the cytoplasm to transfect a cell, lacks oncogenic potential which prevents integration into the genome and acts as an adjuvant by transmitting costimulatory signals through the toll-like receptors.
- Two major factors of the development of mRNA-based cancer vaccines are intrinsic influence and external influence.

fast, customizable, scalable, and low-cost production, mRNA vaccines are reaching their potential as a future crucial strategy for cancer treatment [5].

The nascent clinical use of various innovative cancer vaccines, including immune cell-based, viral vectors-based, and RNA- or DNA-based vaccines, has sparked a lot of interest in cancer vaccines. Among these, the author reported that in comparison to other forms of cancer vaccines, mRNA-based cancer vaccines exhibit extraordinary benefits. This is due to the fact that mRNA-based vaccines are safer and uncontaminated, in contrast to virus-based vaccines which can occasionally be contagious. In addition, the mRNA-carried antigen's genetic information can be rapidly translated into protein after it has reached the cells. On top of that, mRNA-based cancer vaccines can elicit immune response and overcome vaccine resistance, the latter which is frequently seen in cancer treatments using conventional chemotherapies. Additionally, mRNA-based cancer vaccines encode the cancer antigens comprehensively and can thus go through human leukocyte antigen's restrictions to trigger a more extensive immune response. Last but not least, since mRNA cannot integrate into chromosomes, mRNA-based cancer vaccines are free of mutagenic properties. Considering the advantages of mRNA vaccines in cancer management over conventional cancer vaccines, research on this cutting-edge immunotherapy has surged in anticipation of the use of mRNA vaccines for the management of cancer [4].

This review provides a detailed discussion of the emergence of mRNA vaccines in management of cancer which includes: i) the development of mRNA-based cancer vaccine and the process to develop it, ii) the types of mRNA vaccine with the available formulations for *in vivo* delivery, iii) how the immune system responds to the mRNA vaccine for both adaptive and innate immune systems, iv) administration of mRNA-based cancer vaccines, V) clinical overview of mRNA-based cancer vaccines, vi) the advantages of mRNA vaccines over DNA vaccines as well as challenges and opportunities in developing the mRNA vaccines.

2. Methodology

Original and clinical relevance of literature articles published primarily in English are prioritized and non-systematically retrieved from the following databases: Scopus, Google Scholars, PubMed, and ResearchGate. Our search strategy was based on key terms that correspond to the research question: 'mRNA delivery' AND 'cancer,' 'immune response' AND 'mRNA vaccines' AND 'cancer,' 'route of administration' AND 'mRNA cancer vaccine,' 'neoantigen' AND 'mRNA cancer vaccine' AND 'challenges and advances,' 'mRNA design' AND 'mRNA synthesis,' 'mRNA structure,' 'type of mRNA vaccine,' 'cancer vaccine,' 'cancer immunotherapy,' 'mRNA cancer vaccine,' 'mRNA vaccine approval,' 'clinical trials of mRNA,' 'mRNA vaccines combination,' 'mRNA and DNA vaccines,' 'mRNA vaccine formulation,' 'cancer mRNA vaccine,' 'mRNA cancer isolation.' The search process covered all results from 2012 until 2022. Lastly, in the literature search, a total of 52 citations were identified and managed using Mendeley and Zotero.

3. Types of mRNA vaccine

The first mRNA vaccination, which was discovered in 1993, served to target RNA influenza virus. This liposome-entrapped, *in vitro*-transcribed (IVT) mRNA encoding the influenza virus nucleoprotein was effective in inducing virus-specific cytotoxic T lymphocytes (CTL) in mice, which could successfully target and lyse cells infected with the nucleoprotein or the wild type (WT) influenza virus [6]. The discovery and development of mRNA vaccines are still ongoing since then until recently when the booster dose for COVID-19 vaccines manufactured by Moderna and Pfizer-BioNTech are authorized by FDA. The Moderna and Pfizer-BioNTech vaccines have received approval for use as a single booster dose in individuals older than 18 years and older than 12 years of age, respectively. These boosters are from bivalent vaccination type, which comprise two mRNA components of the SARS-CoV-2 virus, one taken from the original strain and the other shared by the Omicron BA.4 and BA.5 lineages of the COVID-19 virus [7]. mRNA vaccine consists of two types: non-replicating mRNA and replicating mRNA.

3.1. Non-replicating mRNA

Differentiating between replicating and non-replicating or self-amplifying mRNA is fundamental. Non-replicating mRNA vaccines consist of the target antigen sequence, 3′ untranslated region (UTR) and 5′ UTR which has a considerably simpler structure. Such vaccines are designed to disintegrate very quickly after producing the antigen as they do not contain any additional genes that encode replication factors. The authors also mentioned that this type of technology is used in the current COVID-19 mRNA vaccines since the transcript can be produced more affordably and integrates more readily inside lipid nanoparticles (LNP) [8].

3.2. Replicating mRNA

In contrast, replicating mRNA contains extra genes that code for self-replication components such as RNA-

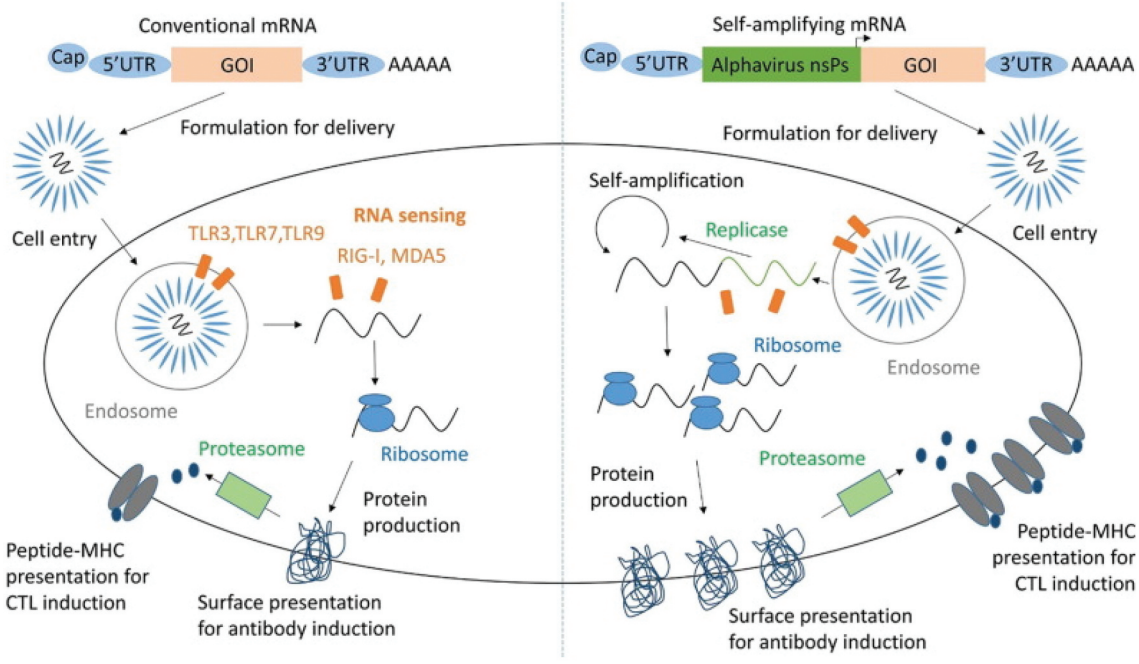

Figure 1. Protein production of saRNA and mRNA in antigen-presenting cells. Taken from [9] under the terms of the Creative Commons Attribution License (CC BY).

dependent RNA polymerase [8]. These innovations generate more transcripts, enabling persistent antigen expression and a prolonged immune response. However, despite numerous recent encouraging research in animal models, they are substantially larger and not yet practical for clinical use. Expression of antigenic protein upon vaccination by mRNA vaccines is presented in Figure 1.

During cancer therapy clinical trials, non-replicating mRNAs are usually studied [2]. However, due to its long-lasting effectiveness and low dosage requirement, self-amplifying mRNA (SAM) or replicating mRNA has received a lot of attention and is being tested in both infectious disease and cancer [2].

There is a type of mRNA vaccine that employs a SAM derived from an alphavirus genome [10]. This type of mRNA contains the genes that encode the replication machinery of the alphavirus RNA but are deficient in the genes which encode the viral structural proteins that are necessary to produce an infectious alphavirus particle. Based on their research, compared to administration of unformulated RNA, SAM enclosed within an LNP significantly boosted the immunogenicity. Therefore, with the protective immune response induced by this SAM, this type of mRNA vaccine was shown to be comparable to those induced by viral delivery technologies [10].

4. Development of mRNA vaccine

4.1. mRNA vaccine design and synthesis

In mRNA-based therapies, mRNA design and synthesis are essential steps. Based on the study conducted by Chaudhary et al., they discovered that the synthetic mRNA molecules used in mRNA vaccines regulate the production of the antigen that will trigger an immunological response [11]. The authors pointed out that the mRNA does not incorporate into the genome, eliminating concerns about genetic modifications, unlike some viral vaccinations. After the pathogen's genome has been sequenced, a target antigen sequence is developed and inserted into a plasmid DNA construct. After that, through bacteriophage polymerase *in vitro*, the plasmid DNA is transcribed into mRNA.

The authors declared that from 5′ to 3,′ the mRNA resembles the structure of endogenous mRNA which is composed of five functional parts. These functional parts consist of 5′ cap, 5′ UTR, 3′ UTR, a frame of open reading that encodes the antigen, and a poly(A) tail. The authors also mentioned that these components act as mediators between mRNA degradation and translation efficiency (Figure 2).

A triphosphate bridge connects the 7-methylguanosine nucleoside in the 5′ cap structure to the 5′ end of mRNA. The 5′ cap additionally protects the mRNA from exonuclease degradation, and together with poly(A) binding proteins, translation initiation factor proteins, and the poly(A) tail at

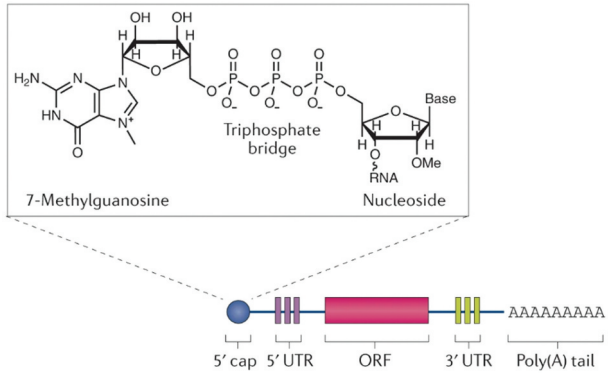

Figure 2. IVT mRNA contains five functional parts: a 5′ cap containing 7-methylguanosine linked through a triphosphate bridge to a 2′-O-methylated nucleoside, flanking 5′ and 3′ UTRs, a poly(A) tail, and an open reading frame (ORF) [11].

the 3′ end, it gets involved with themselves to circulate the mRNA and attract ribosomes to initiate translation [11]. It was also mentioned that for mRNA vaccines to be effective, appropriate 5′ and 3′ UTR sequence design is essential. As a result of the extensive research that has been done to choose and create the best 5′ and 3′ UTR sequences for mRNA vaccines, UTR sequences are regarded as the intellectual property of vaccine developers [12].

The mRNA vaccine's ORF, which contains the coding sequence that is translated into protein, is also its most important component. The mRNA sequence generally contains modified nucleosides to optimize translation, such as N1-methylpseudouridine and pseudouridine. The use of modified nucleosides, particularly modified uridine, blocks pattern recognition receptors (PRRs) from being activated thus allowing for high enough levels of translation to make enough protein as a precaution [12,13].

The fundamental principle of mRNA vaccine technology is based on a way of delivering a molecule of nucleic acid into the target cell in the human host that encodes the desired antigen. Consequently, this enables the host cell to express the antigen and produce the target protein to trigger the immunological response. In this approach, the host's immune system can rapidly initiate humoral and cellular immunological responses against an invasion by an antigen-carrying pathogen, thereby preventing the disease [12].

The specification of the antigen sequence of the target antigen is necessary for the design and manufacture of an mRNA vaccine. The mRNA can be transcribed *in vitro* by RNA polymerase by determining the target antigen and optimizing its coding sequence. After being synthesized, mRNA is purified using a variety of techniques, combined with a lipid phase using microfluidics, and then encapsulated in an mRNA-LNP complex. Soon afterward, the self-assembly of LNPs is finalized by dilution followed by concentrated ultrafiltration. The mRNA vaccine is finally obtained after sterile filtration, filling, loading, and capping [11].

5. Preparation of mRNA-based Cancer Vaccine

5.1. Isolation of mRNA cancer

Various types of cancer vaccines have been developed to combat cancer which include the immune cell-based vaccines, peptide-based vaccines, viral vector-based vaccines, and DNA- or RNA-based vaccines [2]. However, nucleic acid vaccines, especially the RNA-based vaccine, have emerged widely in the development of cancer vaccines [2]. This is due to the fact that this type of vaccine can induce both the humoral and cell-mediated immune responses by covering various somatic tumor mutations that might hinder the effectiveness of the vaccines. Moreover, they also stated that nucleic acid vaccines have a broader T-cell response to overcome the restriction of the human leukocyte antigen (HLA) types by encoding full-length tumor antigens; hence allowing more APCs to be present. Thus, it can be said that RNA-based cancer vaccine or isolation of the mRNA cancer from the nucleic acid is the method of choice in mRNA-based cancer vaccine preparation. The transcription strategy of mRNA vaccines is illustrated in Figure 3.

5.2. Sequence construction

After identifying the antigen of choice of the target protein, several steps of gene sequencing, synthesizing, and cloning into the DNA template plasmid are performed [15]. An mRNA-based cancer vaccine production process is initiated by designing a DNA template or pDNA that consists of the ORF, flanking 5′- and 3′-UTRs as well as a primer binding site that contains available RNA polymerase recognition sites to initiate *in vitro* transcription [14]. Moreover, they also stated that the efficacy of translation of the target protein can be improved by using the codon concurrency that affects the amino acids on the mRNA. The sequence could also be designed *in silico* producing a variety of antigen sequences that have efficient leader sequences, optimal codon usage, increased neutralization, and reduced cross-reactivity [15]. An interesting method that could be used to increase the efficacy of protein expression or mRNA translation is by substitution of rare codons with regularly used identical codons [16]. The UTRs play an important role in regulating the protein expression, rates of degradation and translation of mRNA by interacting with different RNA-binding proteins [14]. It is also stated that the 5′-UTR initiates the translation and formation of preinitiation complexes and stabilizes the mRNA. However, the efficacy of translation can be improved by shortening the length of 3′-UTR. An optimal template sequence with high stability and translating efficacy is ideal in the formulation of mRNA vaccines.

5.3. In vitro transcription (IVT)

IVT is defined as the process of transcribing a designed template strand produced through the sequence construction process to an RNA strand by using the base pairing rule [14]. The recognition of promoter by RNA polymerase will initiate the transcription process. It includes the addition of substances such as modified nucleotides, pseudouridine (Ψ), 2-thiouridine (s2U), 5-methyluridine (m5U), 5-methylcytidine (m5C), and N6-methyladenine (m6A) which will concurrently reduce immunogenicity. This nucleoside modification will initiate after the IVT process where a single nucleotide will be replaced with an analogous modified nucleotide triphosphate at each location (Figure 4) [16].

During the process of *in vitro* transcription, the enzyme RNA polymerase will move along the DNA template until the end [15]. Reducing the amount of magnesium ion concentration and performing the process in a high temperature can reduce the amount of double-strand RNA (dsRNA) that is also produced during IVT [14]. This eases the purification process in the last step.

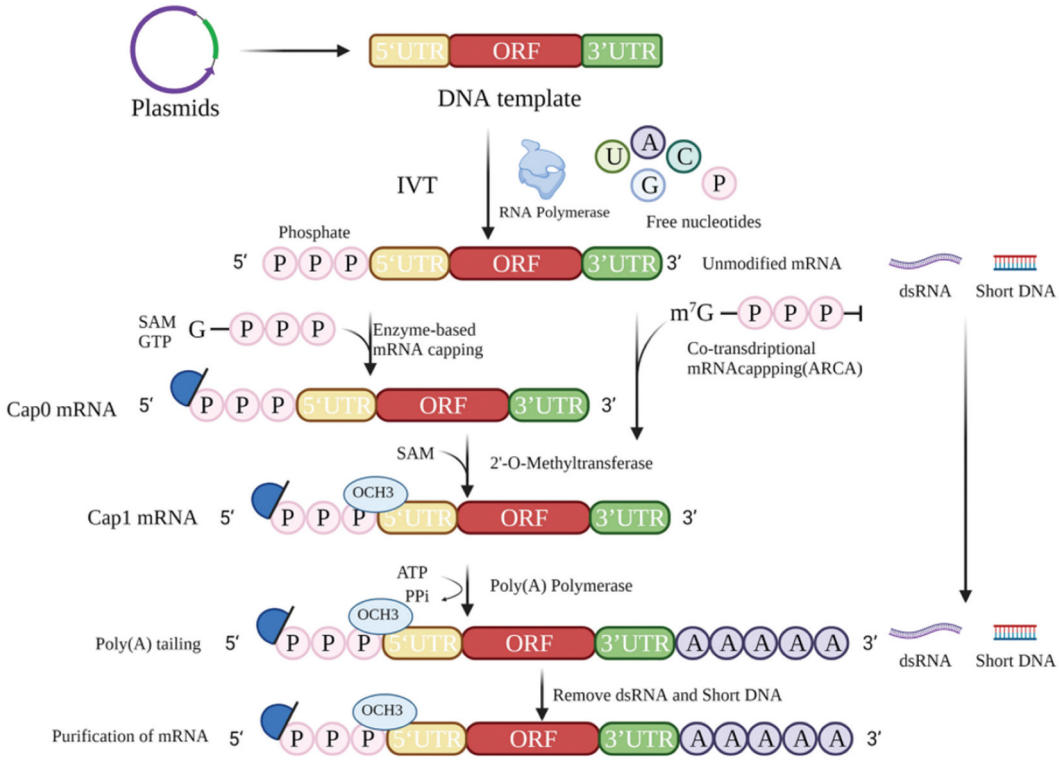

Figure 3. mRNA *in vitro* transcription process consisting of template preparation, *in vitro* transcription, 5′ cap addition, 3′ poly(A) tailing, and purification. Taken from [14] under the terms of the Creative Commons Attribution License (CC BY).

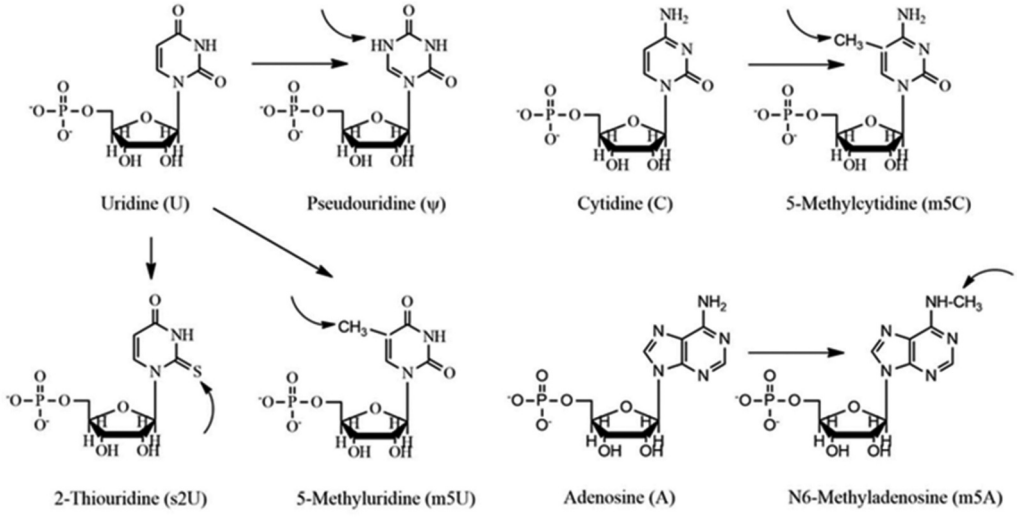

Figure 4. Modified nucleoside bases of uridine (U), cytidine (C), and adenosine (A). Adapted from "Overview on the Development of mRNA-Based Vaccines and Their Formulation Strategies for Improved Antigen Expression in Vivo" [16].

5.4. 5′ cap addition

The synthetic single-strand RNA (ssRNA) produced from IVT is yet to be in its functional structure [14]. Thus, the 5′cap addition process plays a vital role in preventing the RNA being identified as exogenous nucleotides due to the highly immunogenic 5′ triphosphate fraction which may induce type I interferon (IFN-1) response and cause destruction of the RNA protein strand. Thus, replacing the triphosphate fraction with a cap should be done to reduce immunogenicity. 5′ cap structure is important to protect mRNA from intracellular nuclease digestion, producing an increased efficacy when the translation process is occurring on the DNA template [15]. Various methods such as transcriptional and post-transcriptional capping can be conducted to cap the RNA strand. However, it must be noted that capping using these

methods does not cap all the RNA strands entirely [14]. Transcriptional capping is the addition of a cap analogue to the reaction for co-transcription, whereas post-transcriptional capping involves the methylation of Cap 0 to Cap 1 by methyltransferase enzymes. Incorrect capping may increase immunogenicity of exogenous mRNA where it will activate the PRRs and induce IFN-1 response. Therefore, it can be concluded that the success rate is directly proportional to the stability of translation and indirectly proportional to the immunogenicity of the exogenous mRNA.

5.5. Poly(A) tail

The poly(A) tail is important for the ribosomes to access the mRNA sequence as well as its translation efficacy [15]. The poly(A) tail obtained from the addition of poly(A) polymerase or direct transcription is important for the efficiency and stability of mRNA translation by reducing the rate of RNA degradation by RNA exonucleases [14]. They also mentioned that the optimal length of the poly(A) tail depends on the target cell type or can be adjusted optimally by the addition of oligo(dT) in the DNA template. However, the surrounding conditions for the enzymatic reaction, such as temperature and enzyme quality, should be observed in order to correctly produce an optimal length of the poly(A) tail.

5.6. Purification

Transcription of DNA into mRNA usually produces contaminants such as short DNAs and dsRNA due to transcription failure and self-complementary extension, respectively, [14]. These contaminants have a possibility to activate innate sensors which could lead to the destruction of the translated mRNA. Consequently, their removal could reduce these innate immune activation [15]. In order to successfully translate the mRNA and express the encoded protein, purification process should be done to remove the impurities by using high performance liquid chromatography (HPLC) or selective binding of dsRNA to cellulose [14]. Purification using the HPLC method increases the protein expression time due to the dsRNA elimination; hence, lowering IFN-1 and proinflammatory cytokines production [16]. Deng et al. (2022) also stated that mRNA translation and expression highly depend on the purity and sequence composition of the DNA template to produce effective mRNA vaccines [14].

6. Formulation of mRNA Cancer Vaccines

Until recently, the application of mRNA-based cancer vaccines was constricted due to the instability and poor mRNA distribution *in vivo* [17]. Non-formulated mRNA is unstable due to the presence of extracellular and blood RNase [18]. Forchette et al. explained that the negatively charged cell component is electrostatically repulsed by the mRNA's negative charge and preventing it from entering the cell. Therefore, advanced formulations of mRNA-based vaccines are crucial to counter the challenges of delivering mRNA to the target tissue [19].

6.1. LNP formulation

LNP is one of the non-viral vectors for the delivery of mRNA-based vaccines. Its formulations are composed of helper lipids such as distearoylphosphatidylcholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), cholesterol, polyethylene glycol (PEG) lipids, and ionizable cationic lipids [20]. The cationic lipid in the formulation utilizes electrostatic attraction with hydrophobic bonding to encapsulate the naturally negatively charged mRNA which forms complexes [21]. They function to stimulate an immunological response by causing the antibody construction [20]. However, some problems were encountered as cationic lipids were incorporated into the *in vivo* formulation. They are associated with noxious and harmful effects due to their strong positive charge and low endosomal escape in comparison with anionic or neutral lipids [22]. Ionizable lipids for mRNA transport *in vivo* were created to get around these issues by lowering the positive charge, increasing the endosomal escape ability, and lessening immunological and inflammatory reactions that are commonly induced by lipid components [23,24]. The ionizable lipids are neutral at physiological pH which minimizes the possibility of harmful consequences, and they are positively charged at low pH which permits the complexation of negatively charged RNA [19]. These characteristics enable more effective mRNA endocytosis into the cells.

Helper lipids such as PEG are incorporated on the surface of lipid nanoparticles to shelter the cationic lipids, increase the vaccine concentration at the site of action such as liver, overcome *in vivo* limitations, and boost hepatocyte accumulation [25]. Besides, PEG will increase the dispersion of LNP due to the presence of steric barrier around them which consequently hinders LNP from being recognized by the reticuloendothelial system (RES) [19,20]. DOPE, on the other hand, acts differently from other phospholipid by promoting the transport of RNA intracellularly due to its endosmotic or fusogenic function. This is similar to adenovirus or viral vector in that it possesses unsaturated phosphatidylethanolamine, non-bilayer lipid arrangement with membrane fusion [20]. In addition to improving membrane fusion and stability of mRNA vaccine components, cholesterol acts as a neutral lipid that also boosts transfection effectiveness in both *in vitro* and *in vivo* [20].

With the development of scale-up production, mRNA vaccines have a substantial advantage compared to the previous immunization strategies including their quick and inexpensive production [2]. It was explained that, for instance, nearly a million doses of the mRNA vaccine may be produced within a single reaction in a 5-liter bioreactor. Several antigens can be encoded within a single mRNA vaccine, enhancing the immune response against infections with high resistance and allowing the use of a single formulation to target a variety of bacterial or viral strains [11]. Kim et al. mentioned that mRNA was initially not pursued as a therapy, because of the concerns about its stability, ineffectiveness, and overstimulation of the immune system. Fortunately, suitable mRNA structural alterations and formulation techniques have been studied. In the last 10 years,

the use of mRNA in medical settings has reignited research interest in figuring out the mRNA pharmacology, establishing efficient delivery systems, and managing the mRNA immunogenicity [26].

6.2. Lipid/Protamine/mRNA (LPR) nanoparticle formulation

Limitation of naked mRNA for therapeutic use is that the mRNA can be degraded easily by nucleases and the immune response. Multiple components of LPR nanoparticles formulation are responsible to enhance the effectiveness of gene delivery to the tumor site [25]. It has proved that the pharmacokinetics of the vaccine formulation was enhanced with the use of LPR as compared to mRNA without any surface modification. LNP can extend the half-life of free mRNA that usually degrades in the blood before reaching the target tissue and increase the selectivity to the target tissue or tumor due to larger particle size than the liver fenestrae but smaller than the tumor capillary [13]. The polycationic protamine condenses nucleic acid into nanosized complexes to preserve it from nuclease destruction, while the highly coated PEG helps the nanoparticles to escape nonspecific uptake by the RES to prolong circulation duration after intravenous injection [25]. Endosomal escape and release of the mRNA component into the cytosol are made possible by the cationic liposomal membrane, while the addition of low molecular weight anisamide (AA) to the distal end of PEG increases the selectivity toward the cancer cells as the cells overexpress the sigma receptor, making it easier for the nanoparticles to be internalized [25].

6.3. Cell-penetrating peptides (CPPs) formulation

Generally, peptide-based delivery systems offer higher transfection efficiency and reduce harmful effects compared to lipid-based delivery systems [27]. Peptides may have several advantages including biocompatibility, simplicity in synthesis, small size, avoidance of off-target side effects, and attaining the desired effects at lower dosages [28]. CPPs are a non-viral vector type composed of either cationic or amphipathic amino acids which are able to penetrate the cell membrane [26,28]. Large liposomes and bulky molecules can be more easily and effectively internalized into the cell when arginine-rich cationic CPPs are present. These CPP aid in lipid membrane penetration [29] due to their guanidinium group which is responsible for creating hydrogen bonds and electrostatic forces with the cell membrane [26]. Amphipathic CPPs contain both hydrophilic and lipophilic amino acid residues which can interact with neutral and cationic residues of lipid bilayer membrane [30]. Due to their powerful hydrophobic contact and penetration into lipid bilayers of the cell membrane, amphipathic CPPs have a high transfection effectiveness [26]. They also stated that cationic molecules have higher penetration efficiency than anionic molecules as the majority of cancer cell surfaces are generally composed of anionic residues.

7. Immune Response toward mRNA Vaccines

Generally, our cells are given instructions by the mRNA vaccination to produce bacterial or viral proteins. These proteins trigger immune system response, which then creates defenses against infectious pathogens in the future. By using the mRNA vaccine to create antigen in the body, the immune system is prepared when it comes into contact with the real pathogen. Memory B cells rapidly multiply and differentiate to become plasma cells that secrete antibodies in response to a previously known pathogen, ensuring immunization against the desired antigen [31].

After mRNA vaccines are delivered, the immune response will be activated in two different ways: adaptive immune response and innate immune response.

7.1. Adaptive Immune Response

The most crucial cells responsible to initiate the immune response are dendritic cells as the APCs. Initially, mRNA will be endocytosed into the cytoplasm following immunization and bind with the ribosomes in the APCs to initiate and complete the translation process [32]. When the tumor antigen protein has been synthesized, the proteasome will break down the protein into smaller peptides in the cytoplasm and enter the major histocompatibility complex (MHC) presentation cascade [5,32]. MHC will then transport and present the antigenic peptides to cytotoxic T cells on the cell membrane. MHC class I and MHC class II activate the $CD8^+$ T cells and $CD4^+$ T cells, respectively. Besides, dendritic cells also interact with B cells to induce antibody production by the release of antigen proteins from the APCs after they are synthesized. These proteins can be ingested by dendritic cells and presented to the helper T cells and B cells by MHC [32]. Additionally, helper T cells or $CD4^+$ T cells have the ability to co-activate B cells that are specific for an antigen and induce a humoral immune response, while B cells that act as APCs can, in turn, activate $CD4^+$ T cells after internalizing extracellular proteins and presenting on the B cells' MHC class II (Figure 5).

7.2. Innate Immune Response

A self-adjuvant type of mRNA vaccine can initiate the innate immune response after being administered *in vivo*. Initially, APCs identify mRNA, which then activates PRRs which are mostly abundant in the cell's endolysosomal region. PRRs include toll-like receptor (TLR) family such as TLR3, TLR7, and TLR8. mRNA vaccines that are available in the cytosol can be recognized by these receptors [32]. dsRNA triggers the innate immune response by the recognition of TLR3, meanwhile, ssRNA is recognized by TLR7 and TLR8. The downstream pathway activated by TLR7 and TLR8 results in the synthesis of IFN-1 and induces proinflammatory cytokines such as tumor necrosis factor α (TNF-α), respectively [33,34]. The production of IFN-1, proinflammatory cytokines, and other inflammatory molecules increases when APCs engage the downstream pathway, which in turn activates the tumor necrosis factor response [32]. Degradation and inhibition of mRNA translation

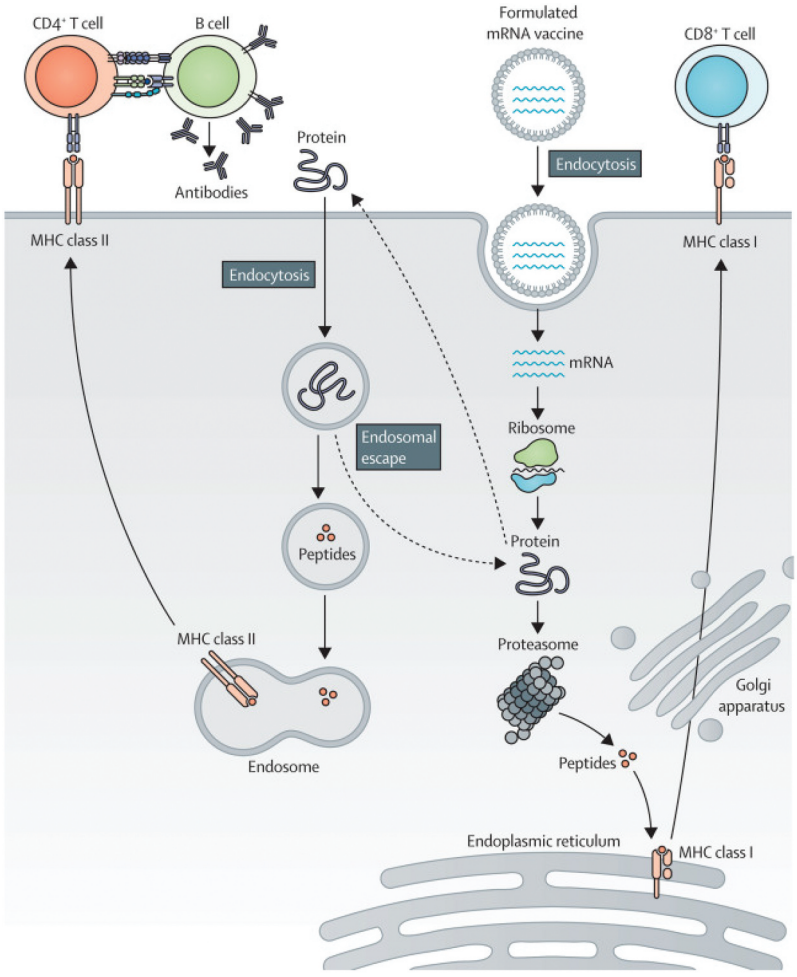

Figure 5. mRNA-based vaccine mode of action for adaptive immune response. Taken from [5] with permission.

can occur as a result of interferons or proinflammatory cytokine release [17]. Both benefits and drawbacks come with the mRNA vaccines' ability to act as their own adjuvant as it can unintentionally inhibit the mechanism of adaptive immune response toward the vaccines [32].

8. Cancer immunotherapy

Cancer immunotherapy affects the immune system by directing it against cancer cells with the goal of ultimately destroying them or preventing their proliferation [35]. Antigens derived from tumor cells are used in cancer vaccines, which are intended for administration to cancer patients. A multitude of methods and forms can be used to administer them. After they are delivered, the host immune system processes the cancer antigen and presents it to effector cells, specifically $CD8^+$ T cells and $CD4^+$ T cells, both of which are crucial parts of adaptive immune response [35].

There are two types of cancer vaccines, which are preventative vaccines and therapeutic vaccines. Vaccines that target viral infections linked to cancer formation were among the first developed to prevent malignancies effectively. The chance of developing hepatocellular carcinoma (HCC) is increased by the hepatitis B virus, a major cause of chronic liver disease. Meanwhile, as immunotherapeutic tools, therapeutic cancer vaccines are utilized to treat diseases that are already active. In cancer immunotherapy, only two therapeutic vaccines have received approval. These include Sipuleucel-T (Provenge), a dendritic cell (DC)-based vaccination for the treatment of castration-resistant prostate cancer, and the Bacillus Calmette-Guérin (BCG) vaccine indicated for early-stage bladder cancer [36]. An autologous cellular immunological drug, Provenge, works by enhancing T cells immunity against the target antigen prostatic acid phosphatase (PAP) which is highly expressed in the majority of prostate cancer cells [37]. Leukapheresis is used to extract patient's peripheral blood mononuclear cells, which are subsequently cultivated *ex vivo* using a fusion protein comprising PAP and granulocyte-macrophage colony-stimulating factor (GM-CSF). After being reinfused three times at an interval of roughly 2 weeks, the product, which comprises activated APCs, induces T cells activation and proliferation as well as antigen-specific reactivity against PAP [38].

Early cancer vaccination therapies relied on self-antigens known as TAAs, which are abundantly expressed on tumor cells. Despite the fact that TAAs-based vaccine therapy was thought to be a novel immunotherapy technique, clinical trials of TAAs-based cancer vaccines for inducing anti-tumor immune response did not show effective results. This is likely because central and peripheral immune tolerances inhibit the activity of TAAs-specific T cells. Additionally, TAAs-based

vaccines are insufficiently selective since TAAs could also express in healthy cells, leading to adverse vaccination reactions such as autoimmune diseases [39]. Therefore, a cancer vaccination needs to be capable of 'break tolerance' by enabling the low-affinity, TAA-reactive T cells that are still present. The activation and multiplication of self-antigen-reactive T cells have been amplified using potent adjuvants, co-stimulators, and repeated vaccinations; these are crucial for the low-affinity T cells. Even with such improvements, the elicited immune response, while detectable, appears to be insufficient for many TAAs-directed vaccine clinical trials to achieve considerable efficacy. When compared to successful antiviral vaccinations, which often produce >5% antigen-specific $CD8^+$ T cells, such vaccines frequently promote the activation and proliferation of antigen-specific $CD8^+$ T cells to a level of only 1% of the total circulating $CD8^+$ T cells. The next target of therapeutic cancer vaccination is tumor-specific antigens (TSAs). TSAs are made up of neoantigens encoded by cancer mutations and antigens released by oncoviruses that are genuinely tumor-specific, allowing high-affinity T cells to exist and be highly stimulated by these antigens [40]. On the viability of mRNA vaccines that target tumor-specific antigens, numerous preclinical and clinical studies have been initiated. As reported by Sebastian et al., the RNActive® vaccine CV9201 for example, induced an antigen-specific immune response in 65% (30/46) of stage IIIB/IV non-small cell lung cancer patients. This led to stable disease in 31% of evaluable patients and treatment-free survival in 5 stage IV patients for more than a year [34]. Knowledge of how early generation vaccine target is also important for us to be able to tackle arising problems for the improvement of later generation vaccines in achieving patients' immunity.

9. Cancer antigen types

Every kind of cancer has its very own type of antigens. It is crucial to know how types of antigens differ from one another by distinguishing their advantages and disadvantages and creating a list of available antigens that can be related to every type of cancer. Thus, it is important to implement this strategy to select the most compatible antigen for mRNA cancer vaccine. TSAs are the ideal targets in designing the mRNA-based cancer vaccines which include immune-privileged antigens, neoantigens, and viral antigens [41].

Immune-privileged antigens are one of the antigen types that can adapt widely toward the spectrum of malignant diseases with its high specificity and low central tolerance. Moreover, it can also be produced in large amount due to its well-established manufacturing technologies by identifying its whole gene and amino acid sequence. This will then lead to a more affordable mRNA-based cancer vaccine treatment. However, some disadvantages also exist. Certain cancer vaccine such as PReferentially expressed Antigen in MElanoma (PRAME)-based cancer vaccine could potentially induce autoimmune response. Tumor heterogeneity also limits the efficacy of immune-privileged, antigen-based vaccines.

Like immune-privileged antigens, neoantigens also induce high specificity antitumor immunity and low central tolerance. It also has a high affinity toward HLA and T-cell receptors. Hence, a personalized strategy can be provided which overcomes tumor heterogeneity in each patient suffering from the same type of disease. However, the process is time-consuming where it takes at least 2–3 months to design and expensive due to complex procedures and limited preparation techniques.

Lastly, viral antigens also have high specificity and low central tolerance where they could trigger immune response against virus-related tumors. Furthermore, it requires simple preparation procedures which lead to well-established manufacturing technologies. However, it is limited only toward virus-related cancers and the efficacy could be reduced if any mutations occur in the viruses [42].

10. mRNA-based cancer vaccine administration

10.1. mRNA-based cancer vaccine dosage

Various clinical trials have been conducted by researchers in finding the best recommended dose of mRNA-based cancer vaccine. Kübler et al. has shown a phase I/IIa study of prostate cancer patients receiving a prostate cancer vaccine CV9103 containing self-adjuvanted mRNA. It was stated that the recommended dose for prostate cancer vaccine was 1280 µg [43]. However, side effects such as fatigue, chills, and injection site reactions were also present after the treatment. Another study conducted by Yang et al. has shown that a lipoplex-mRNA vaccine had an effective expression toward claudin (CLDN) 6 or CLDN6-LPX in *ex vivo* human dendritic cells with a dose of 100 µg/ml. However, more research must be done to produce a more efficacious and tolerable mRNA-based cancer vaccine with fewer side effects and affordable to patients in the real-world clinical setting [44].

10.2. Route of Administration

In general, there are various administration routes of mRNA vaccines that can elicit different effects in infectious disease and cancer. The most commonly used routes to administer vaccine are intramuscular (IM), subcutaneous (SC), intradermal (ID), and intravenous. These routes are known as parenteral as the administration routes used are not via the digestive tract.

These parenteral routes have different effectiveness in activation of immune cells as different injection sites would have different levels of immune cells, APCs, and DCs. Based on an independent study, subcutaneous and intramuscular routes displayed no difference in antibody activation. Theoretically, administration via subcutaneous route should elicit higher level of immunogenic effect than intramuscular due to the fact that dermis contains higher number of immune cells compared to muscle tissue [45]. For example, when HIV gp140 surface glycoprotein-encoding saRNA vaccination formulated with LNP based on the ionizable lipid DLin-DMA was tested for its immunogenicity with different route of administration, IM route is more effective when compared with the ID and SC routes but there was no significant difference between IM and ID routes. In another experiment, HA-mRNA-LNP vaccine was used, and HAI titers were considerably higher in ID route than IM route. However, 2 weeks after vaccination boost, it was found that both administration routes had similar yields

[46]. In addition to the parenteral route, nebulization of mRNA encapsulated in 1,2-Dioleoyl-3-trimethylammonium propane-based LNP was demonstrated in an animal study. The study found that the nebulization approach had no influence on the mRNA integrity or the efficiency of encapsulation [47].

11. Clinical Overview of mRNA Vaccines for Cancer

There are two factors that are predominant in mRNA-based cancer vaccine development: intrinsic influence and external influence [48]. The intrinsic influence deals with the alteration of the mRNA molecule itself, therefore affecting the mRNA-based cancer vaccine's effectiveness. This is done through enhancing mRNA structure and sequences, progressing mRNA preparation and purification technologies, and creating new delivery vectors. On the other hand, the external influence deals with central tolerance to tumor antigens, tumor heterogeneity and HLA186, and tumor immune microenvironment [48]. In terms of these tumor and HLA heterogeneity factors, the development of tumor neoantigen vaccines has become a major focus for cancer vaccine research because of their stronger specific antitumor effects and lower toxic side effects than TAAs-directed vaccines. Furthermore, researchers have developed an immune-based combination for dealing with tumor microenvironment factors. This is accomplished by combining mRNA cancer vaccines with adjuvants or immune checkpoint inhibitors such as anti-PD-1, anti-CTLA-4, and anti-PD-L1 antibodies, which has emerged as a major trend in the application of mRNA-based cancer vaccines. The promising clinical treatment responses across cancer diagnoses have been shown due to the use of therapeutic mRNA cancer vaccines in conjunction with other immunotherapeutic treatment methods such as immune checkpoint inhibitors, oncolytic viruses, and adoptive cell therapy, in association with the highly immunosuppressive tumor microenvironment [5]. For instance, advanced melanoma patients received a combination therapy known as TriMixDC-MEL IPI, which combined ipilimumab and immunization with DC-based mRNA encoding TriMix and tumor antigens. As they were able to elicit potent tumor-associated antigen-specific CD8+ T cell responses, the tumor-specific vaccination and immune checkpoint blockers showed significant therapeutic benefits [49]. Though many clinical studies have been tested on people with diverse cancer kinds, such as pancreatic cancer, colorectal cancer, and melanoma for mRNA vaccine therapy and also for combination of vaccine with medicine, the US Food and Drug Administration still has not yet licensed any mRNA cancer vaccine for use either on its own or in combination with other cancer treatments [50].

12. mRNA vaccine advantages over DNA vaccines

The question raised now is whether mRNA cancer vaccines are promising? Across the journals reviewed, mRNA cancer vaccines are very promising for possible cancer prevention platform because they have a high potential for overcoming the current challenges [2,51]. McNamara et al. also emphasized the advantages of mRNA vaccines over the DNA-based vaccines. Because of the inefficiency of DNA delivery into human cells, DNA vaccines elicit a weaker immune response than other types of vaccines. They also have the potential to cause cancer by integrating into the host's genome. On the other hand, mRNA vaccines are superior to DNA vaccines where they retain the same appealing properties and advantages as DNA vaccines. To begin its action, RNA only needs to enter the cytoplasm where translation occurs. Furthermore, because RNA cannot integrate into the genome, it lacks oncogenic potential. Finally, RNA can act as an adjuvant by transmitting costimulatory signals through the toll-like receptors TLR3, TLR7, and TLR8 [52]. With all the benefits considered, it is clear that there is a strong desire to expand research on mRNA cancer vaccines.

13. Challenges and Opportunities in Developing mRNA Vaccines

Research on mRNA cancer vaccines is quickly advancing as time goes on since these vaccines have demonstrated remarkable efficacy in the therapeutic treatment of cancer. This has been demonstrated by numerous effective preclinical analyses of various cancer kinds. Although mRNA cancer vaccines have advanced quickly, there have not been many new problems encountered, which has created numerous prospects for advancement. There are two challenges and opportunities that have been a major research study which are the route of administration and neoantigen.

13.1. Route of administration

Route of administration is one of the challenges in developing mRNA vaccines. The major concern in the route of administration is the ability to evaluate the most practical vaccination delivery methods [5]. Delivery methods such as intranodal, intradermal, subcutaneous, intramuscular, intravenous, and intratumoral administration are frequently used for mRNA cancer vaccines (Figure 6). Each method has benefits, and their drawbacks.

Among the challenges related to the route of administration is that different administration routes have different impacts on a vaccine's efficacy. This is because different parts of the body injected will have different amounts of antigen presenting cells and immune cells [48]. For example, the subcutis region, into which the vaccine is subcutaneously injected, contains less antigen presenting cells than the dermis since it is mostly made up of a loose network of adipose tissues. Moreover, compared to conventional administration methods, large amounts can be injected subcutaneously, because the skin is elastic and allows for simultaneous use of several injection sites. However, the volume of injections per site should still be kept to a minimum because high quantities may cause the skin to stretch painfully [54]. Hence, this has great advantage as it allows for a higher injection volume via the subcutaneous method, in which this subcutaneous region also has fewer local adverse effects.

Intranasal route of immunisation is advantageous due to its amenability for repeated administration, non-invasive nature and high patient compliance [55]. Moreover, because the nasal mucosa is rich in antigen presenting cells and immune cells, it

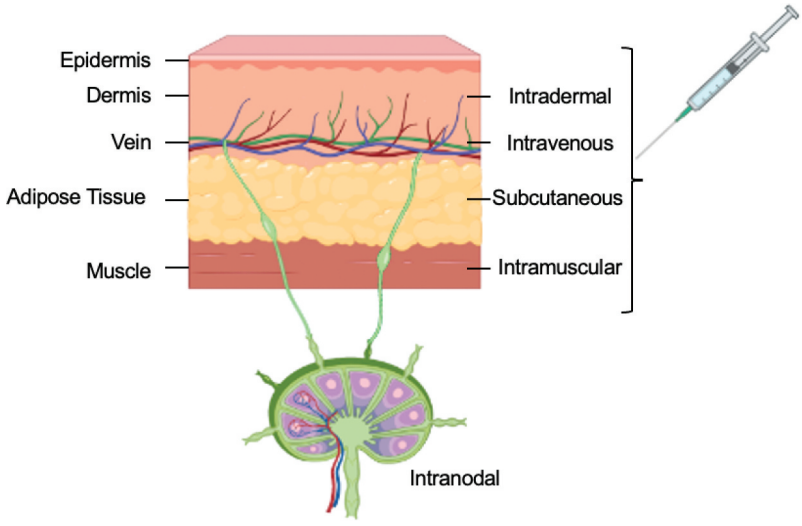

Figure 6. The routes of delivery for mRNA vaccines. Taken from [53] under the terms of the Creative Commons Attribution License (CC BY).

has been proven as the intranasal administration of lysophosphatidylcholine mRNA (LPC-mRNA) and intranasal administration of mRNA nanoparticle vaccination can both trigger antitumor immune responses. Preclinical studies have shown the preliminary efficacy of mRNA cancer vaccines administered by intranasal injection [48]. However, studies of intranasal mRNA vaccines have been restricted to preclinical animal models and it is likely that further development of lipid nanoparticle carriers will be necessary to effectively target the right cell types in the upper respiratory tract [56]. It is also mentioned that it is effective in neutralizing infectious respiratory viruses like SARS-CoV-2 by using an inhaled or intranasal vaccine that may elicit both cellular and humoral responses.

In comparison with intranodal, intranodal is an unconventional yet effective vaccine delivery that involves intranodal infusion of naked mRNA. The intranodal injection concentrates the vaccine components at the tissue region where naive T and B cells are primed, but this advantageous colocalization is transient because afferent lymph quickly flushes the lymph nodes [57]. Hence, it will cause disadvantages as the quality and longevity of the immunological memory produced by vaccines may be limited by too quick clearance of the vaccine, as prolonged antigen over many days seems to enhance adaptive immune responses. Although it has this disadvantage, but in a research by Bol et al., it can be seen that patients with advanced melanoma can be safely vaccinated therapeutically with an autologous mRNA-optimized DC vaccine intranodally. However, due to a few tumor-associated antigen-specific immunological and clinical responses were seen intradermal or intravenous injections are more likely to be favored due to their technical simplicity. As a result of the success of preclinical investigations, clinical trials using intranodally injected naked mRNA expressing tumor-associated antigens in patients with advanced melanoma and patients with (HCC) have been started. Consequently, the research on route of administration is crucial and requires deeper research findings due to variances in the efficacy of various routes of administration [58].

Moreover, the same type of mRNA vaccine can have different effects on the immune system, depending on the route of administration. For instance, the route of administrations of mRNA-lipoplex determines the opposing effects of type 1 interferon signaling on the extent of vaccine-evoked T Cell responses [59]. When the mRNA-lipoplexes are administered intravenously, it resulted in high-magnitude T cell responses and produced significant antitumor effectiveness. When the mRNA-lipoplexes administered subcutaneously, they were connected to a significantly increased cytolytic CD8 T cell response while previously it has lack of type I interferon signaling. Moreover, intravenously injected an mRNA cancer vaccine candidate, which is delivered as an RNA-lipoplex formulation and encodes a fixed combination of four tumor-associated antigens such as MAGE-A3, NY-ESO-1, TPTE, and tyrosinase that are common in melanoma, induces persistent and potent antigen-specific CD8 T cell responses as well as objective responses in patients with unresectable melanoma [48]. This cancer vaccine has been given FDA fast track designation for clinical translation to treat advanced melanoma in light of these findings. Moreover, the route of administration is also associated with the risk of side effects. Between systemic delivery and local injection of mRNA vaccines, local injection has a low risk of side effects as this method of delivery aims to inject directly into the organ or tissue that is the target [53].

13.2. Neoantigen

Another opportunity that can be explored is the identification of individual cancer neoantigens. Neoantigens are abnormal peptides that malignant cells particularly express on their surfaces. Neoantigens may come from the open reading frames of viral genomes and be seen in tumors that are linked to viruses, such as cervical cancer. mRNA vaccines have become a promising platform for cancer immunotherapy. During vaccination, naked or vehicle loaded mRNA vaccines efficiently express tumor antigens in antigen-presenting cells (APCs), facilitate APC activation and innate/adaptive immune stimulation. mRNA cancer vaccine precedes other conventional vaccine

platforms due to high potency, safe administration, rapid development potentials, and cost-effective manufacturing. However, mRNA vaccine applications have been limited by instability, innate immunogenicity, and inefficient in vivo delivery. Appropriate mRNA structure modifications (i.e. codon optimizations, nucleotide modifications, self-amplifying mRNAs, etc.) and formulation methods (i.e. lipid nanoparticles (LNPs), polymers, peptides, etc.) have been investigated to overcome these issues. Tuning the administration routes and co-delivery of multiple mRNA vaccines with other immunotherapeutic agents (e.g. checkpoint inhibitors) have further boosted the host antitumor immunity and increased the likelihood of tumor cell eradication. With the recent U.S. Food and Drug Administration (USFDA) approvals of LNP-loaded mRNA vaccines for the prevention of COVID-19 and the promising therapeutic outcomes of mRNA cancer vaccines achieved in several clinical trials against multiple aggressive solid tumors, making envision the rapid advancing of mRNA vaccines for cancer immunotherapy in the near future. This review provides a detailed overview of the recent progress and existing challenges of mRNA cancer vaccines and future considerations of applying mRNA vaccine for cancer immunotherapies [2]. Moreover, neoantigen-based cancer treatments are ground breaking, in which chemotherapy and radiation therapy treatment cannot do, because neoantigen will program the immune system to exclusively kill cancer cells that exhibit neoantigens, leaving all other cells in the body unaffected. Challenges of using neoantigens are that the clinical translation is limited by the difficulties of antigen predictions and the suboptimal immunogenicity and difficulties in identifying and efficiently delivering highly immunogenic tumor-specific antigens.

The neoantigen's immunogenicity is influenced by a number of different factors in addition to its structure. Neoantigens are acquired by antigen-presenting cells, such as dendritic cells and macrophages, after being released by cancer cells. The proteasome breaks down neoantigens into tiny peptides, which are then loaded onto major histocompatibility complex molecules and displayed on the surface of antigen presenting cells. Then, neoantigen-loaded antigen presenting cells go into lymph nodes that drain tumors. Specific T cells identify neoantigen-major histocompatibility complexes by T cell receptors when the proximity of antigen presenting cells and T cells is convenient for interaction. As a result, the spatial location of the antigen presenting cell is another critical factor.

One of the challenges in neoantigen is that tumor antigens are highly variable across different individuals, and some are less immunogenic and can invade the recognition by the host immune system. Even if the antigen is immunogenic, a suppressive microenvironment could prevent effective T cells' infiltration and cause T cell exhaustion [2]. Moreover, it has been a challenge in the ability to identify individual cancer neoantigens. Years of research exploring mRNA vaccines for cancer treatment in preclinical and clinical trials have set the stage for the rapid development of mRNA vaccines during the COVID-19 pandemic. Therapeutic cancer vaccines based on mRNA are well tolerated, and the inherent advantage in ease of production, which rivals the best available conventional vaccine manufacture methods, renders mRNA vaccines a promising option for cancer immunotherapy. Technological advances have optimized mRNA-based vaccine stability, structure, and delivery methods, and multiple clinical trials investigating mRNA vaccine therapy are now enrolling patients with various cancer diagnoses. Although therapeutic mRNA-based cancer vaccines have not yet been approved for standard treatment, encouraging results from early clinical trials with mRNA vaccines as monotherapy and in combination with checkpoint inhibitors have been obtained. This Review summarizes the latest clinical advances in mRNA-based vaccines for cancer treatment and reflects on future perspectives and challenges for this new and promising treatment approach [5]. In a recent study in 2021, on 729 breast cancer patients, patients who were reported to have a 'high degree of neoantigen expression' showed increased survival [60]. Even though the number of survivors is not mentioned in the study, a modest number of survivors suggests the necessity to generate antigen for a large number of patients. However, this seems impossible as the variability of neoantigens in individuals is so large that their immunogenicity fluctuates according to the genetic instability of cancer cells. Although there are challenges in neoantigen, neoantigen-based vaccines have been shown promising efficacy as it has proven to be effective in mouse models for cancers such as skin, colon, and bone cancers [61]. In summary, the highly variable across different individuals has become a challenge in implying neoantigen in cancer vaccines, but due to promising efficacy found in animal clinical trials, further research is needed to fully utilize the newly found findings.

In short, despite these difficulties, the encouraging study of a new generation of cancer medicines based on the understanding of neoantigens is aimed at reducing negative side effects and increasing tumor selectivity. Cancer patients will soon have a superior quality of life that is essentially pain-free because these procedures will not require any hazardous chemicals or damaging radiation.

14. Conclusion

In conclusion, according to WHO (2022), cancer is the leading cause of death worldwide in 2020, accounting for over 10 million deaths, or nearly one in every six. Hence, with the knowledge of the cancer prevention, prevalence, and the development of a variety of cancer, the opportunity to reduce cancer prevalence becomes possible.

15. Expert Opinion

In general, the two types of mRNA vaccines currently in development are non-replicating mRNA and replicating mRNA. Non-replicating mRNA vaccines have the benefit of transcript being able to be produced more economically and integrated into lipid nanoparticles more easily. Replicating mRNA vaccines, on the other hand, can produce more transcripts, allowing for longer immune response and sustained antigen expression. The steps involved in mRNA-based cancer vaccines formulation are isolation of cancer mRNA and transcription strategy which includes the process of sequence construction, *in vitro* transcription, 5′ Cap addition, poly(A) tailing, and

purification. Several formulations of mRNA vaccines utilizing lipid nanoparticles, lipid/protamide/mRNA nanoparticles, and cell-penetrating peptides have been developed to overcome the challenges in delivering mRNA to target tissue. In order to create better vaccine technology, the interaction between immune cells and cancer cells needs to be understood first. There have been many clinical studies on the feasibility of cancer vaccine for cancer immunotherapy, however no such vaccines have been approved by the FDA. With the approval of booster doses for COVID-19 vaccines from Moderna and Pfizer-BioNTech, we are seeing progress toward the discovery of an mRNA-based cancer vaccine with the goal of improving the intrinsic and external influence of the cancer vaccine. Given the advantages of mRNA-based vaccines over DNA-based vaccines, we are optimistic that mRNA-based vaccines will be useful in cancer management in the future. Finally, two challenges in mRNA vaccine development have been highlighted which are the route of administration and neoantigen. These challenges present opportunities for further mRNA vaccine development. Nevertheless, there are numerous encouraging studies of new generation cancer medicines that can lead toward improved prognosis and better quality of life for cancer patients.

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