RBCBM Revista Brasileira de Ciências Biomédicas

MESSENGER RNA VACCINE AS CANCER IMMUNOTHERAPY: CURRENT ADVANCES AND FUTURE PERSPECTIVES

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ABSTRACT

Messenger ribonucleic acid (mRNA) has shown itself as a promising vaccine platform, especially after the success of the COVID-19 vaccine, developed by Pfizer-BioNTech and Moderna and approved for use in 2021. Such enthusiasm occurred because mRNA vaccines present low cost and have the possibility of rapid and large-scale production, besides its capacity of encoding various different antigens. In this sense, there has been an increase in interest in the usefulness of mRNA vaccines as cancer immunotherapy, considering that there is the possibility of encoding individualized tumor antigens, which can be associated or neoantigens, that will be delivered to antigen-presenting cells (APCs), inducing humoral or cellular immune response. Thus, several research groups are developing mRNA vaccines against different cancers, in order to elaborate an effective and safe treatment for cancer patients. However, some negative points of this technique include mRNA instability, high cost for producing patient individualized antigens and the inefficient antigen delivery of naked RNA due to its low half-life *in vivo*, needing the help of a delivery system. In this review, we will discuss how the mRNA is made and how it activates the immune response, highlighting its advantages and disadvantages, besides its existing delivery systems, with lipid nanoparticles in focus (used in the COVID-19 vaccine) and some developing clinical trials.

Keywords: mRNA vaccine; immunotherapy; cancer.

INTRODUCTION

Messenger ribonucleic acid (mRNA) is a product of transcription used in translation, serving as a carrier of genetic information from desoxyribonucleic acid DNA for protein synthesis via the ribosome. It was discovered in 1961 by Brenner et al. [1,2,3]. However, its use as a vaccination agent only proved successful in 1990, when Wolff et al. obtained protein expression through mRNA transfection in mice [4]. Although the use of mRNA as a vaccine agent was proposed in 1990, the delay in advancing studies to ensure vaccine approval was due to the fact that RNA is a highly unstable molecule, exhibits high immunogenicity, and initially presented limitations in delivery systems. Over the years, extensive research has been conducted to optimize the molecule [2,3,4].

In 1796, Edward Jenner introduced the first concept of vaccination by inoculating healthy individuals with microorganisms that cause cowpox, observing that they developed immunity against human smallpox. Since then, vaccines have evolved from attenuated pathogens to subunit vaccines, recombinant vaccines, and, more recently, mRNA-based vaccines [1,5]. In this regard, despite existing challenges, messenger RNA was conceived as a vaccine antigen due to the need for vaccines that can be produced rapidly and on a large scale, unlike other current vaccine platforms [2]. Consequently, the effectiveness of rapid mRNA vaccine production was demonstrated in 2020 during the SARS-CoV-2 pandemic, the agent of COVID-19. In January 2020, the viral genome sequence was published, and mRNA vaccines, encoding the viral Spike protein, developed by Moderna and

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DOI: https://doi.org/10.46675/rbcbm.v7i1.113. Article received: April 2, 2026; accepted: April 10, 2026; published: April 17, 2026 in the Brazilian Journal of Biomedical Sciences, available online at www.rbcbm.com.br. All authors contributed equally to the article. The authors declare no conflict of interest.

Pfizer-BioNTech were approved in December 2020 in the United States for population-wide use, with a development timeline of less than one year [1,4].

Since then, researchers have increasingly focused on the development of other messenger RNA vaccines, with particular emphasis on anti-tumor therapy. This area of study is of great importance, as cancer is considered one of the greatest challenges faced by medicine in this century [6]. It is estimated that this disease was responsible for approximately 1,670 deaths per day in the United States in 2023 and about 10 million deaths worldwide annually, indicating high mortality and morbidity, with limited curative outcomes despite existing treatments such as chemotherapy, radiotherapy, and surgery [6,7]. In this regard, the effectiveness of alternative anti-tumor approaches becomes essential in order to improve patient outcomes. With this perspective, in the late 19th century, William Coley was the first to manipulate the immune system using live and inactivated bacteria in cancer patients, leading to tumor remission, and establishing the foundation for the concept of cancer immunotherapy [8].

Immunotherapy is currently considered a major innovation in cancer treatment, particularly following the success of chimeric antigen receptor T cell (CAR-T cell) therapy. It functions by activating the patient's immune response, modifying the tumor microenvironment, and promoting the destruction of cancer cells. Similarly, vaccines utilize antigens to elicit an immune response, which can be cellular (mediated by T lymphocytes) and humoral (mediated by B lymphocytes). The first tumor vaccine was developed in 1988 using tumor antigens derived from an allogeneic melanoma cell line, eliciting an anti-melanoma response. This approach has since evolved into vaccines currently available, such as those against cervical cancer, caused by Human Papillomavirus (HPV), and gastrointestinal cancers associated with Hepatitis B Virus (HBV) infection. In this context, tumor vaccines can act both prophylactically, by generating immunological memory, as seen with HBV and HPV vaccines, and therapeutically, as a form of immunotherapy for existing cancers, inducing a sustained immune response against tumors. These vaccines can be developed from cells, peptides, viruses, or nucleic acids [8,9]. In this review, we describe how mRNA vaccines can be used as a cancer immunotherapy, its effects on the immune system and tumoral microenvironment, its advantages and disadvantages and analyze and compare ongoing studies.

METHODS

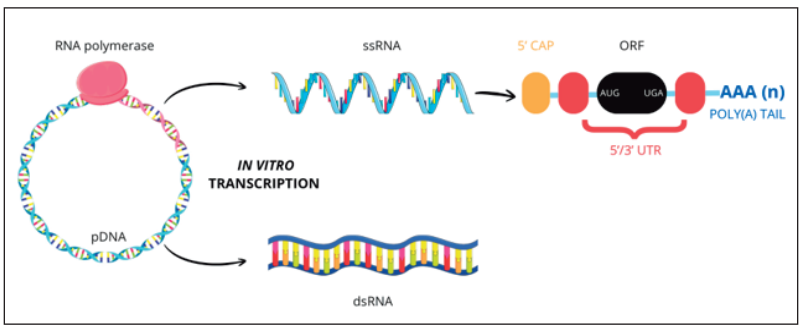
This study is a qualitative narrative literature review of a descriptive nature, aiming to explore discussions in scientific publications and relevant clinical trials regarding advances in messenger RNA vaccines, with a particular focus on their application as cancer immunotherapy. In this context, articles indexed on PubMed and Google Scholar databases, in English from the past five years, that is, from 2020 to 2025, using the descriptors: "mRNA," "vaccine," "cancer," "delivery," "trials," "advantages" and "disadvantages" were selected. As exclusion criteria, articles published before 2020, those without full-text access, and those not specifically focused on the use of messenger RNA as a vaccine agent in cancer immunotherapy were excluded, except those used in Section 3.1.

DISCUSSION

Technique

The most advanced and currently used technique for manufacturing mRNA as a vaccine antigen is *in vitro* transcription (IVT), in which a linearized plasmid DNA (pDNA) or a synthetic DNA prepared by Polymerase Chain Reaction (PCR), containing the gene of the desired antigen, is used, along with the addition of T3, T7, or SP6 RNA polymerase to ensure proper transcription [1,3]. This reaction produces several products; however, the main ones are double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA), the latter being the antigen required for vaccine production (see Figure 1).

Figure 1: *Representation of in vitro transcription of messenger RNA from a plasmid DNA containing the sequence of the antigen to be encoded, generating ssRNA and dsRNA. The ssRNA structure includes a 5' cap, a 5' UTR, a coding region (ORF), a 3' UTR, and a poly(A) tail.*



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 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

The ssRNA requires specific structural elements in its composition to maintain its functionality, ensuring molecular stability and the proper translation needed to generate the desired antigen. To achieve this, it must contain a 5′ cap, 5′ and 3′ untranslated regions (UTRs), an open reading frame (ORF), and a poly(A) tail [1,2,3,5]

5' Cap

The 5′ cap is responsible for eliminating free phosphate groups to increase mRNA stability, as well as enabling the ribosome to recognize the start of the strand through the eukaryotic translation initiation factor 4E (eIF4E), thereby improving translation efficiency. In addition, the incorporation of anti-reverse cap analogs (ARCA) prevents reverse incorporation of the 5′ cap, further enhancing translation efficiency [3]. Moreover, in 2023, Gote *et al.* highlighted studies indicating that this element also plays a role in distinguishing *in vitro* mRNA from endogenous mRNA, thereby inducing an immune response. However, to avoid triggering an excessive immune response, the 5′ cap hinders the activation of retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) receptors, preventing the inhibition of translation [1].

5' e 3' UTRs (untranslated regions)

Untranslated regions do not directly participate in translation; however, they can influence its efficiency and are therefore used to ensure mRNA stability and optimal translation, similarly to the 5′ cap. They are located at both the 5′ and 3′ ends of the *in vitro* mRNA strand, and their optimization is essential for vaccine efficiency.

The 5′ UTR is essential to prevent improper initiation of translation, ensuring that the base sequence present in the ORF produces the desired antigen protein. Additionally, to stabilize the mRNA molecule and improve translation, specific sequences may be added to this region. The 3′ UTR, in turn, is located in a relatively unstable region and can be optimized by incorporating stabilizing elements while avoiding A–U and G–U sequences, which are considered unstable [3]. Therefore, untranslated regions play a key role in controlling the rate of translation and increasing mRNA half-life [2], as well as regulating gene expression and enhancing ribosomal recognition of mRNA to improve translation efficiency [1].

ORF (open reading frame)

The ORF is the coding region of *in vitro* mRNA, where the codons responsible for generating the antigen of interest are located, and its optimization depends on different strategies. Optimization of this region involves incorporating synonymous codons and/or codons with high transfer RNA (tRNA) abundance in order to improve translation and ensure antigen expression. However, optimization is not always beneficial, depending on the desired antigen and the proteins required for its expression, as some proteins rely on low-expression codons [3].

Furthermore, the ORF is what differs between conventional mRNA vaccines and self-amplifying (saRNA) mRNA vaccines: while conventional vaccines have a small and simple structure, with only a single ORF region — which helps prevent an undesired immune response, saRNA vaccines contain a replication gene, similar to viral genes, along with a gene encoding the therapeutic antigen, enabling RNA self-replication and its translation into the vaccine antigen [2].

***Poly*(A) Tail**

The poly(A) tail is a region located at the 3′ end of the mRNA, consisting of a sequence of adenine (A) nucleotides. It is added to the mRNA to reduce strand degradation by RNA endonucleases. This region is responsible for increasing mRNA stability, extending its half-life, by increasing the length of the tail, and enhancing the efficiency of translation into the desired antigen [1,3,5].

According to Xu et al., the addition of the poly(A) tail to mRNA occurs through its binding protein, poly(A)-binding protein (PABP), which can interact with the 5′ cap via translation initiation factors, thereby increasing strand stability and improving translation. However, this protein can also bind to adenylation complexes and inhibit microRNA (miRNA)-mediated translation. Finally, it is also known that recombinant poly(A) polymerase can be used to add poly(A) structures through enzymatic polyadenylation after the initial transcription [3].

Immunogenicity

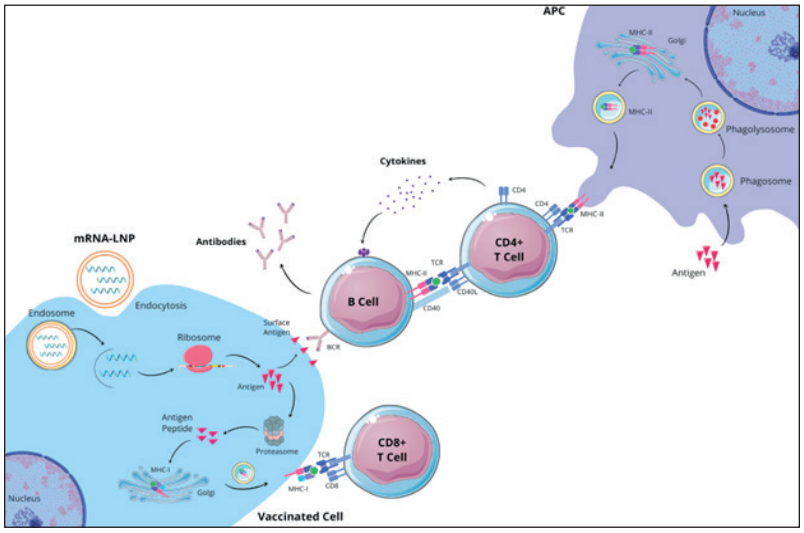
The *in vitro* mRNA manufacturing technique primarily produces two types of RNA: a single-stranded RNA (ssRNA) and a double-stranded RNA (dsRNA), which act differently upon undergoing endocytosis by non-immune cells or antigen-presenting cells (APCs). Endocytosis occurs for both RNA types due to their virus-like properties, allowing their recognition by these cells as a result of their self-adjuvant effect. This characteristic also enhances anti-tumor reactivity, thereby increasing T cell responses [2]. Within the endosome, both molecules are recognized by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) — TLR3 for dsRNA, and TLR7 and TLR8 for ssRNA, modulating the immune response according to the type of mRNA [2,3].

Double-stranded RNA activates TLR3, an innate immune response receptor present in the cellular cytoplasm, enabling dsRNA to bind to receptors such as RIG-I, MDA5, among others. This interaction induces the maturation of APCs, as well as the secretion of pro-inflammatory cytokines and type I interferon (IFN). However, an excessive response can induce genes that inhibit the translation of ssRNA by releasing antiviral enzymes that promote its degradation, since dsRNA is recognized by the same receptors as non-self RNA, making optimization necessary to ensure effective vaccine delivery. In contrast, single-stranded RNA activates TLR7 and TLR8, innate immune receptors located in endosomes, triggering antiviral responses in dendritic cells and stimulating nitric oxide synthesis, also inducing the secretion of type I IFN [2,3].

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

Following the aforementioned process, after the endocytosis of ssRNA by a non-immune cell, it is recognized by ribosomes, where translation of the messenger RNA into the required antigenic protein occurs. In this context, this protein may be expressed by the non-immune cell as a surface antigen, being recognized by B lymphocytes, which, in addition to producing antibodies, also present it via Major Histocompatibility Complexes (MHCs), a set of genes that encode proteins responsible for antigen identification and presentation, through class II MHC for CD4+ T lymphocytes, which secrete pro-inflammatory cytokines. Furthermore, the produced proteins may also be secreted by the non-immune cell, being recognized by B lymphocytes and also endocytosed by antigen-presenting cells (APCs), where they undergo lysosomal degradation to generate peptides that will be presented via class II MHC to CD4+ T lymphocytes, removing them from the naïve state. Finally, the protein may also undergo proteasomal degradation within the non-immune cell, generating peptides that will be presented via class I MHC to CD8+ T lymphocytes [1,5]. In this way, vaccination becomes effective by activating both humoral and cellular immune responses — cytotoxic and helper — as illustrated in Figure 2.

Figure 2: *Representation of messenger RNA translation by a vaccinated cell and antigen presentation to APCs. In this image, the mRNA is associated with a lipid nanoparticle (LNP) and is endocytosed by the cell, followed by the release of the LNP and escape from the endosome due to their degradation by compounds present in the lipid nanoparticles. Thus, the mRNA is read by the ribosome, generating the required antigen. Protein antigens may be degraded by proteasomes and activate CD8+ T lymphocytes (cytotoxic) through class I MHC receptors. In addition, extracellular antigens released by the vaccinated cell are taken up by APCs, degraded, and presented to CD4+ T lymphocytes, which are activated and secrete cytokines that stimulate B lymphocytes to produce antibodies against the antigen. Finally, protein antigens can be presented as a surface antigen and activate B lymphocytes.*



Source: *Figure created by the author using Servier Medical Art (https://smart.servier.com) and Bioicons.*

Mechanism of Action

Messenger RNA vaccines aim to eliminate tumor cells by inducing an adaptive (long-lasting) immune response against the tumor through the encoding of tumor-specific antigens (TSAs), tumor-associated antigens (TAAs), or immunomodulatory factors, functioning as a therapeutic approach for existing cancer [6,7,8]. This treatment is more specific than chemotherapy, radiotherapy, or surgery, as it inhibits tumor growth and reduces the likelihood of metastasis and recurrence, considering that this mechanism activates both humoral and cellular immune responses, thereby improving the patient's quality of life [7,8].

Thus, the manufacturing process of mRNA vaccines begins with obtaining a patient sample, followed by its sequencing to identify the required tumor antigen, this may include tumor-specific antigens, tumor-associated antigens, or immunomodulatory factors, mRNA production (either conventional, with the addition of an adjuvant, or self-amplifying RNA (saRNA), with the inclusion of viral RNA for additional replication, ensuring longer-lasting efficacy with fewer doses [3], and finally vaccine administration [6,9]. T lymphocytes are the main mediators of the anti-tumor response; therefore, they are the primary targets of the vaccine to achieve effective treatment, requiring the occurrence of three signals in the T cell [10].

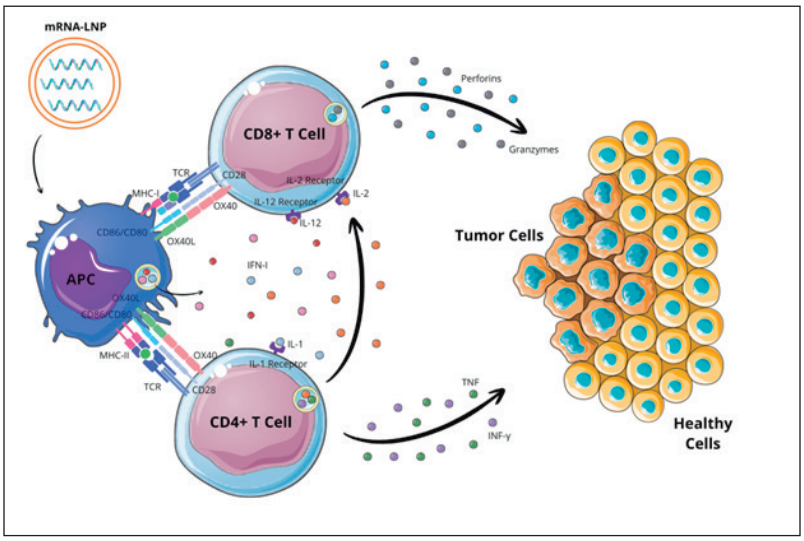
After the antigen is produced from the mRNA, it is presented via class I and class II MHC on the surface of APCs: class I MHC presents endogenous antigens to activate CD8+ T lymphocytes, thereby triggering a cytotoxic response; whereas class II MHC presents exogenous antigens, activating the helper response— this interaction represents the first signal for cellular immune response activation and T cell proliferation. The second signal occurs through co-stimulatory molecules CD80, CD84, and OX40L

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

binding to CD28 and OX40 receptors on T cells. The third and final signal arises from type I interferons and interleukins (IL)-12 and IL-1 binding to cytokine receptors on T lymphocytes [10].

In addition, CD4+ T lymphocytes are responsible for the secretion of IL-2, an interleukin that acts as a stimulator of CD8+ T lymphocytes, further enhancing their proliferation and generating a strong CD8+ T cell response. Thus, after activation and proliferation, both helper and cytotoxic T lymphocytes migrate to the tumor microenvironment and release chemokines to maximize the action of cytokines such as IFN-γ, tumor necrosis factor (TNF), granzymes, and perforins, which are responsible for killing tumor cells [10], as illustrated in Figure 3.

Figure 3: *Representation of the action of humoral and cellular immune responses on tumor cells through antigen presentation by APCs. In the image, the first signal of lymphocyte activation is depicted via class I and class II MHC, the second signal through co-stimulatory molecules CD80, CD84, and OX40L binding to CD28 and OX40 receptors, and finally, the third signal through IL-12 and IL-1 signaling, along with type I IFNs. Thus, cytokines and chemokines are released in order to attack tumor cells.*



Source: *Figure created by the author using Servier Medical Art (https://smart.servier.com) and Bioicons.*

Tumor-Specific Antigens (TSAs)

Tumor-specific antigens (TSAs), also known as neoantigens, as the name suggests, are specific to tumor cells: they are present only in cancer cells, as they arise from mutations, thus, they can be used to identify whether a mutation has occurred. Because they are not present in healthy cells, these antigens are highly immunogenic and elicit a robust T cell response due to low immune tolerance, while also causing minimal damage to healthy cells because of their low toxicity to the organism [6,7,8,10].

According to a study from The Cancer Genome Atlas, there is a correlation between the number of neoantigens and the expression of genes related to CD8+ T lymphocyte activity: the more mutated the tumor, the higher the expression of surface markers such as CD8A, PD-1, and CTLA-4 on mature T lymphocytes, leading to increased patient survival due to greater T cell infiltration into the tumor microenvironment [8,10].

Additionally, He et al. report that when there are around 10 somatic cell mutations, the likelihood of TSA formation increases, whereas tumors with fewer than one somatic mutation show a reduced probability. Rajasagi et al. also demonstrated that TSAs are common in most tumors by analyzing mutant human leukocyte antigens (HLAs) across 13 tumor types. Thus, TSAs, being unique to each tumor and patient, are excellent antigens for vaccine expression via messenger RNA due to their high immunogenicity, high specificity, and low self-tolerance [8,10].

Associated-Tumor Antigens (TAAs)

Tumor-associated antigens (TAAs) are antigens present in cancer-related genes, germline genes of healthy cells, or specific differentiation markers, meaning they are expressed by both tumor cells and normal cells [6,7]. Despite this characteristic, these antigens can still be used to elicit an immune response because they are overexpressed in cancer cells, allowing the generation of mRNA based on this overexpression [6,8].

However, even though TAAs are overexpressed in tumor cells, the fact that they are also expressed in healthy cells poses a challenge for their use: they can elicit immune responses against both normal and tumor cells, resulting in limited success due to potential autoimmune toxicity to healthy tissues [6,7]. Moreover, because they are normally expressed antigens, the body may recognize them as self, even indicating low immunogenicity, high tolerance, and limited specificity [8,10]. Therefore, it is ideal to use multiple tumor-associated antigens in combination to generate an effective antitumor response, along with the addition of an adjuvant

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

to optimize vaccine performance [10].

Immunomodulation

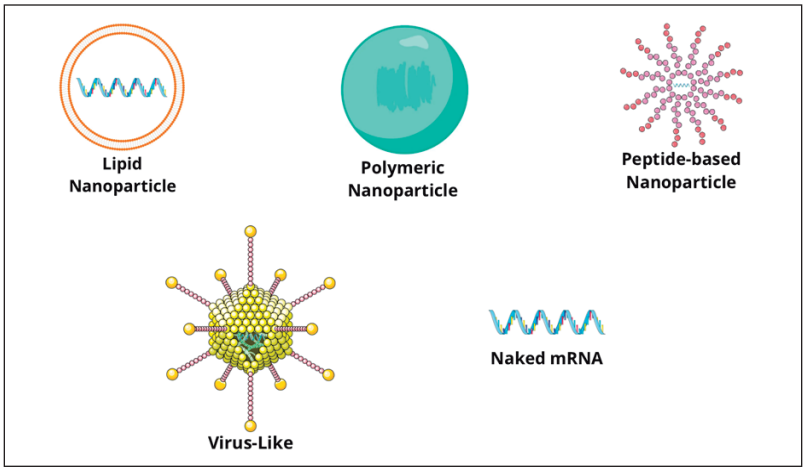
Immunoregulatory factors are molecules capable of stimulating or suppressing immune cell functions, such as cytokines and receptors. In this context, messenger RNA is responsible for encoding these factors in order to elicit an immune response, inducing them simultaneously; the most common include IL-2, IL-12, and OX40L, previously mentioned as stimulators of T lymphocyte activation and proliferation, thereby enhancing the antitumor response. Furthermore, immunoregulatory factors can also function as the adjuvants previously mentioned in relation to TAAs, promoting an increased immune response and facilitating their recognition by APCs [6,7].

Delivery System

For the vaccine to be effective, IVT mRNA must be delivered into the cellular cytoplasm of a cell at the injection site or into an antigen-presenting cell [7,12,13]. However, it is a high–molecular weight, negatively charged, and unstable molecule, which is easily degraded by endo- and exonucleases, such as RNase. This makes it difficult for IVT mRNA to cross membranes, results in a short half-life, and leads to poor cellular uptake, ultimately hindering its delivery to the cytosol [7,12,13,14]. Furthermore, in the context of cancer vaccines, there is the additional challenge of an immunosuppressive tumor microenvironment, making efficient delivery of the antigen to dendritic cells (DCs) critically important [14].

Therefore, the use of delivery systems becomes essential, as they are responsible for making the vaccine safer, more stable, and more efficient *in vivo*, protecting IVT mRNA from RNases, increasing its transfection efficiency, and reducing its size through lipid nanoparticles (LNPs), polymer-based nanoparticles, peptide-based nanoparticles, and virus-like delivery systems, which will be described later. Moreover, in the context of cancer, delivery systems can ensure specific targeting to the tumor microenvironment, reducing adverse effects and enhancing antitumor immunity [9,10,15]. Common examples of delivery systems are illustrated in Figure 4.

Figure 4: *Representation of different types of delivery systems. In order: LNP, polymer-based nanoparticle, peptide-based nanoparticle, virus-like system, and naked mRNA.*



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Lipid Nanoparticles (LNPs)

Lipid nanoparticles are molecules that can act as delivery systems for messenger RNA as a vaccine antigen in order to protect it from degradation and increase its efficiency, ensuring that translation occurs successfully. For this purpose, LNPs are composed of a membrane made of phospholipids and ionizable lipids, with the addition of cholesterol and polyethylene glycol (PEG), and an aqueous core where the mRNA is contained [7,9,10,14]. Let us discuss all the properties of this compound.

The ionizable lipid functions to form a stable lipocomplex with the mRNA, aiding in fusion with the endosomal membrane and facilitating the escape of messenger RNA into the cellular cytoplasm. In this context, it is designed to remain neutral at physiological pH (approximately 7.4) and become protonated within the endosome (approximately pH 6.5), allowing the lipocomplex to bind electrostatically to the cellular membrane and promote membrane fusion, also increasing the compound's half-life in the bloodstream and reducing its toxicity. Additionally, protonation facilitates endosomal escape into the cytosol through electrostatic interactions with the membrane and its subsequent disruption [7,9,12,14].

The lipocomplex membrane is also composed of phospholipids, which assist in the fusion of the LNP with the cellular membrane by mimicking it; thus, the immunogenicity of the delivery system is reduced by resembling self-components, while also contributing

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

to its structural integrity [7,10,12]. Moreover, cholesterol is also present in the LNP membrane, functioning to stabilize the molecular structure by modulating the membrane in a physicochemical manner [7,10,14].

Finally, polyethylene glycol is included, a hydrophilic polymer that influences circulation time and cellular uptake, being responsible for increasing the half-life of the lipocomplex, typically used at 1–3% to enhance delivery. However, caution is required with higher amounts of PEG, as it is an immunogenic molecule that may induce the production of anti-PEG antibodies, leading to an immune response upon administration of a second vaccine dose. In summary, the ionizable lipid nanoparticle remains neutral in circulation and, upon reaching the target cell, undergoes endocytosis. Within the more acidic environment of the endosome, it becomes protonated and interacts electrostatically with the target cell membrane, enabling membrane fusion and disruption. This process allows the mRNA to successfully reach the cellular cytoplasm and be translated [7,10,14].

In view of this, LNPs are considered the most widely used non-viral delivery system and the most clinically advanced, with approximately three generations of LNPs developed to reach those used today. The first generation was not biodegradable, making the compound highly toxic; after optimization, the second generation incorporated biodegradable ester linkages, although these were limited to carrying small mRNAs; finally, the third generation improved the transfection of larger mRNAs and is currently used in vaccines such as those for COVID-19 [12,14,15].

In this context, lipid nanoparticles have undergone continuous optimization to become versatile, minimally immunogenic, suitable for large-scale production, and applicable to prophylactic, immunostimulatory, and therapeutic vaccines. They also exhibit low toxicity and are biodegradable, thereby increasing biocompatibility [7,9,14]. Nevertheless, there is still room for improvement: at high doses, LNPs may cause side effects such as anaphylactic and inflammatory reactions, and they can be hepatotoxic, although this can be mitigated by targeting strategies, such as coupling with adjuvants to direct them to specific cells [7,14].

A research group led by Chen et al. developed LNPs specifically designed to act in lymph nodes against liver, spleen, and lung cancers, achieving reduced accumulation of lipid nanoparticles in the liver and increased accumulation in lymph nodes through subcutaneous administration. In this context, vaccinated mice exhibited a strong CD8+ T cell response and developed immune memory, without progressing to tumor metastasis. Thus, these findings indicate a promising outlook for tumor vaccines using LNPs as a delivery system, although further clinical trials are still required.

Polymeric Nanoparticles

Polymeric nanoparticles are molecules that are typically cationic, hydrophilic, and effective carriers of messenger RNA for *in vitro* transfection, and they can be composed of polyamides, dendrimers, or biodegradable copolymers. However, positively charged polymers such as polyethyleneimine (PEI), despite being the most commonly used polymeric delivery system for nucleic acids, are high–molecular weight molecules, which hinders their clearance and indicates low biodegradability, making them highly toxic [9,12].

From this perspective, other types of polymers have been developed for mRNA delivery systems, such as poly(β-amino ester) (PBAE) and poly(lactic-co-glycolic acid) (PLGA), which are designed to be less toxic than PEI by increasing biodegradability. Zhang et al. developed a polymeric nanoparticle combining PBAE with poly(glycolic acid) (PGA) that carries mRNA encoding transcription factors to reprogram macrophages with tumor-associated antigens, promoting an anti-tumor phenotype against melanoma, glioblastoma, and ovarian cancer. Also, PLGA has been shown in studies to be stable, minimally toxic, and to provide controlled release; however, due to its anionic nature, it does not efficiently encapsulate mRNA at physiological pH, making it less effective than ionizable LNPs, for example, despite its tolerable toxicity [12,14].

Although studies are ongoing and more complex polymeric systems are being developed, polymeric nanoparticles are not as clinically advanced as LNPs [9]. According to Wadhwa et al., research on polymers is more empirical than definitive due to the body's biological response to them, with lipid nanoparticles being 100 to 1000 times more efficient than polymer-based systems. As a result, research efforts are much more focused on LNPs as delivery systems for messenger RNA vaccines.

Peptide-based Nanoparticles

In turn, this delivery system is composed of low–molecular weight, positively charged peptides that, upon electrostatic interaction with mRNA, condense into nanoparticles, such as protamine. These systems exhibit high versatility and biocompatibility, as they are composed of proteins such as arginine, lysine, among others; however, they present low target specificity and a short half-life due to their limited stability [9,12,14].

An example of peptide-based nanoparticles is protamine, as previously mentioned: in addition to its properties that protect messenger RNA from RNase degradation, it is primarily used as an adjuvant, activating TLR7 and TLR8, which are responsible for inducing a Th1-type response, a pro-inflammatory pathway. Moreover, protamine can also be combined with LNPs, providing both mRNA condensation into nanoparticles through the peptide and the presence of an ionizable molecule with a high capacity for membrane fusion [9,14].

Finally, cationic cell-penetrating peptides (CPPs) have also been developed; due to their positive charge, they interact electrostatically with the anionic cellular membrane, facilitating binding to glycosaminoglycans on the cell surface and promoting micropinocytosis. These molecules are efficient in delivering cargo to dendritic cells to induce T cell responses; however, there are still few studies involving their use with mRNA [9,14].

Virus-Like

Adeno-associated viruses, such as alphaviruses,

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

picornaviruses, and flaviviruses, can be genetically modified, with their genes being partially or completely replaced by model genes or therapeutic genes in order to be used as delivery systems for genetic material. These viruses are cytopathogenic, invading the target cell, replicating, and being expressed in the cytoplasm due to their nature as positive-sense single-stranded RNA viruses, that is, they possess a genomic sequence that can be directly translated into the protein of interest by host ribosomes. Although this is a promising delivery system, it is limited by host anti-vector immunity, leading to high immunogenicity due to cytotoxicity and eventual rejection by the host [7,12].

Route of Administration

One of the most important factors to consider when analyzing the efficiency of vaccine antigen delivery is the route of administration. Although the optimal route depends greatly on the type of tumor and the patient, several options are available when considering mRNA vaccines for tumors: intravenous, intradermal, subcutaneous, intramuscular, intranodal, and intratumorally administration, each with its own advantages and disadvantages [10].

Intravenous vaccine administration allows for the injection of larger volumes of the vaccine content and enables direct delivery to lymphoid organs through circulation, making it more likely to induce a CD8+ T cell response. However, for the same reason, it carries a high risk of systemic toxicity and activation of the innate immune response. In contrast, intradermal vaccination has the advantage of delivering the vaccine more directly to APCs, particularly within lymphatic vessels and connective tissue; however, the administered volume must be reduced due to a higher incidence of adverse reactions [9,10].

In addition, there are also subcutaneous and intramuscular routes of administration: the former involves a site with few APCs but abundant adipose tissue, resulting in fewer adverse effects; the latter is the most commonly used for vaccines in general, allows flexible volume administration, and, due to its high vascularization, promotes significant recruitment of immune cells, also leading to fewer adverse reactions compared to other routes. Both routes mentioned above involve immune cell infiltration; however, subcutaneous administration is more immunogenic at the injection site, whereas in intramuscular administration the antigen is transported to the lymph nodes [9,10].

There is, finally, intranodal administration, which, despite targeting a site rich in antigen presenting cells, is technically challenging, and intratumorally administration, which is more commonly used for immunostimulatory molecules, requires lower volumes, and is also inherently complex due to the nature of the site [9,10].

In conclusion, for messenger RNA vaccines against cancer, intramuscular, subcutaneous, and intradermal administration are the most commonly used, immunogenic, and long-lasting routes, although they are applied in different volumes depending on the specific injection site [7,9].

Clinical Trials

A research group led by Chen et al. (2022) developed a lipid nanoparticle named LNP 113-012B. This LNP is specifically designed to be delivered to lymph nodes, acting as a delivery system in a therapeutic mRNA vaccine for lung, spleen, and liver cancers. The developed mRNA encodes the ovalbumin (OVA) protein with the aim of enhancing CD8+ T cell responses and generating a therapeutic effect against the B16F10 tumor model, which expresses OVA. In addition, the mRNA can also encode the tumor-associated antigen TRP2 to further enhance its therapeutic effect, and it is combined with an anti-programmed death-1 (anti-PD-1) antibody to boost the immune response.

Thus, Chen et al. induced the B16F10 tumor in mice and divided them into three groups: control mice (non-vaccinated), mice vaccinated with the 113-012B vaccine, and mice vaccinated with the ALC-0315 vaccine (a COVID-19 vaccine developed by Pfizer-BioNTech). The results presented below refer to the mice vaccinated with 113-012B, developed by the research group.

As previously mentioned, one of the major limitations of LNPs as a delivery system is the high proportion of the compound metabolized by the liver, which may cause damage. By specifically targeting lymph nodes, this study aimed to reduce liver accumulation and increase lymph node expression through subcutaneous administration of the vaccine. This effect was confirmed through bioluminescence, comparing organs from mice vaccinated with 113-012B and ALC-0315.

Additionally, through flow cytometry, it was demonstrated that the antigen was successfully delivered to APCs, activating the adaptive immune response and generating a rapid pro-inflammatory response, with total IgG antibodies detected after 14 days (measured by ELISA), along with high levels of IL-6, associated with T cell responses, and IFN-γ, the latter persisting for more than four weeks after the second dose. Finally, the vaccine also induced an increase in M1 macrophage responses (pro-inflammatory) near the tumor, a reduction in regulatory T cells (Tregs), likely due to the addition of anti-PD-1, and an increase in CD8+ T cells.

Based on the aforementioned tests, the vaccine proved to be safe *in vivo* and suitable for clinical application, as all surviving mice vaccinated with 113-012B did not develop new nodules after recovery (no tumor recurrence) and showed no progression to lung metastasis, indicating the development of immune memory against the cancer. Furthermore, the vaccine demonstrated excellent protective efficacy, confirmed for more than 40 days, with no tumor growth observed in vaccinated subjects. Despite these promising results, 113-012B is still under investigation and has not yet been approved for clinical use.

On July 26, 2023, Moderna and Merck announced their phase 3 vaccine for melanoma in patients following oncologic surgery: mRNA-4157, composed of a messenger RNA responsible for encoding 34 different patient-specific antigens and the monoclonal antibody pembrolizumab (anti-PD-1). The study includes 1,089 patients, with results expected by 2029. In this context, during

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

phase 2, patients who received the vaccine showed a 44% lower risk of tumor recurrence or death compared to treatment with pembrolizumab alone. Furthermore, no increase in pembrolizumab-related adverse effects was observed, and all vaccine-related adverse events were grade 3 or lower, with the most common being fatigue, injection site pain, and chills.

Considering that the encoded antigens are patient-specific, the process from patient sample collection to mRNA development takes approximately 6 weeks, with the goal of reducing this to 30 days. After vaccine production, it is administered intramuscularly in 9 doses, one every 3 weeks, along with 9 doses of pembrolizumab administered intravenously.

There is also a vaccine developed by Cafri, Robbins, Rosenberg et al. (2020), named mRNA-4650, for metastatic gastrointestinal cancers. It consists of a messenger RNA encoding more than 20 different neoantigens and a support structure containing all mutations in the TP53, KRAS, or PIK3CA genes, along with approximately 15 human leukocyte antigen class I neoantigens to bind to the patient's MHC.

Thus, four patients with metastatic gastrointestinal cancer were treated with mRNA-4650, administered intramuscularly in four doses, with two-week intervals between them. The development of the personalized vaccine took approximately 42 to 60 days. The results showed no severe adverse effects and no clinical response in three out of four patients, although all exhibited CD4+ and CD8+ T cell responses to the neoantigens expressed by the vaccine. However, the vaccine did not proceed to phase 2 trials because, in addition to the lack of clinical efficacy, Moderna is currently conducting clinical trials with a vaccine combined with anti-PD-1 therapy.

Finally, another research group led by Rojas et al. (2023) is developing an mRNA vaccine that encodes individualized neoantigens (up to 20 per patient), with the addition of atezolizumab (an anti-PD-1 monoclonal antibody), cevumeran (an mRNA vaccine containing more than 20 class I and II MHC-related neoantigens), and mFOLFIRINOX (a chemotherapy regimen) as adjuvants, aiming to serve as immunotherapy for pancreatic cancer (PDAC). This vaccine uses LNPs as its delivery system and was administered intravenously to PDAC patients following oncologic surgery. Its main objective is to amplify neoantigen-specific T cells that were previously inhibited by PD-1 and to activate these T cells against the neoantigens, considering that survivors of this adenocarcinoma typically develop T cell responses against neoantigens.

15 patients under the previously described conditions were vaccinated, and the results demonstrated that the vaccine is safe, feasible, and capable of generating neoantigen-specific T cell responses in 50% of patients. Moreover, findings indicated that T lymphocyte expansion may persist for more than 10 years after vaccination, suggesting that, despite PDAC being a low-mutational cancer, the vaccine can induce T cell responses against the required neoantigens, although it remains unclear whether these are CD8+ or CD4+ T cells.

It was concluded that patient-specific neoantigens can be identified within approximately 9 weeks and integrated into treatment after oncologic surgery, respecting a period of more than 12 weeks post-surgery. This demonstrates that the vaccine is capable of inducing long-term T cell memory responses in post-surgical pancreatic cancer patients, with reduced chances of recurrence.

Advantages and Disadvantages

Although messenger RNA vaccine technology has only recently begun to receive proper attention, it is a highly promising immunotherapy approach, with several advantages compared to other types of vaccines, such as DNA-based and attenuated vaccines. mRNA vaccines allow for rapid, large-scale, and cost-effective production due to their short development cycle and the fact that they do not require cell culture, making it possible to produce them in approximately three weeks. From this perspective, these vaccines are also highly versatile, enabling personalized therapeutic approaches by using patient-specific antigens, as discussed in the previous section [6,7,8,9,10].

Moreover, an important factor in its production is that, as a messenger RNA, the genetic information is translated more rapidly into protein, and can be translated in any cell containing ribosomes, occurring in the cytoplasm [10,19]. Due to this characteristic, in contrast to DNA vaccines, mRNA does not exhibit mutagenic properties; that is, it is not capable of integrating into the chromosome, resulting in a low genetic risk and avoiding oncogenic effects associated with genetic mutations [6,7,8,9,10,19]. Furthermore, another advantage of this molecule is its ability to encode a complete antigen or multiple different antigens, enabling APCs to present a variety of HLA epitopes, thereby overcoming their restrictions [9,19].

Since the molecule used in the vaccine does not contain an actual pathogen, this immunotherapeutic approach is considered safe, with a low risk of viral infection and free from contamination [6,7,8,9,10,19]. Finally, considering that tumor immunotherapy vaccines can be personalized, they become more effective, better tolerated (due to low toxicity), and more specific, leading to improved treatment responses. In this way, mRNA is capable of stimulating a strong immune response, overcoming the resistance commonly observed in chemotherapy, and generating both humoral and cellular antitumor responses of high magnitude [6,7,8,9,19].

In contrast, naked RNA is an unstable molecule, highly susceptible to degradation by RNases in the extracellular environment, and is therefore not efficiently internalized by APCs, resulting in low vaccine efficiency. Due to its rapid degradation, this molecule exhibits a short half-life and ineffective *in vivo* delivery, factors that further contribute to its limited efficacy [8,10,19]. It is also known that messenger RNA is an immunogenic molecule, capable of activating innate immunity through interferons (IFNs) due to impurities such as double-stranded RNA (dsRNA) generated during *in vitro* synthesis, which reduces the amount of circulating mRNA and may lead to adverse effects in patients [8,9,19].

Finally, when considering its use as a carrier of individualized

 DOI: https://doi.org/10.46675/rbcbm.v7i1.113

tumor antigens, the identification of these antigens can be a time-consuming and costly process, and changes in the patient's condition may occur during the time required for vaccine production [10].

Optimization

As previously mentioned, the immune response induced by mRNA, particularly the secretion of type I interferons, is strong against specific antigens and may lead to the production of antiviral enzymes or RNases that degrade messenger RNA, thereby blocking translation and reducing vaccine efficacy. Thus, it is important to optimize the molecule in order to improve vaccine efficiency and effectiveness *in vivo*. This can be achieved by developing RNAs that are less sensitive to RNases, such as synthetic RNAs or mRNA analogs like self-amplifying RNA, which increases protein production by self-replication, or even circular RNA, which can evade responses triggered by pathogen-associated molecular patterns (PAMPs), thereby preventing molecular degradation [9,19].

Furthermore, impurities can be removed from messenger RNA to make it free from contaminants and avoid non-specific activation. Optimization of ORF regions, as well as 5′ and 3′ UTRs, can also be employed to enhance protein expression by improving translation efficiency and stability [8,20]. Ultimately, it is essential to integrate delivery systems into the vaccine to ensure efficient mRNA delivery to APCs, particularly through the use of LNPs, peptides, and other systems previously mentioned [9,19].

CONCLUSION

Messenger RNA vaccines represent a highly promising vaccine platform with great potential to transform how vaccines are developed and applied, both in the context of tumors and infectious diseases. Their efficacy is evident in the ability of mRNA to induce both cellular and humoral adaptive immune responses, activating T lymphocytes and promoting antibody production by B lymphocytes through antigen presentation by APCs. Moreover, mRNA is an extremely versatile molecule, capable of encoding tumor-associated antigens, tumor-specific antigens, or even immunomodulatory molecules to enhance the patient's own immune response against the tumor, such as cytokines and chemokines, thereby enabling the development of personalized vaccines tailored to an individual's tumor. mRNA vaccines offer several advantages, including rapid and large-scale production, as they are generated through *in vitro* transcription of plasmid DNA without the need for cell culture, as well as a low mutagenic potential due to their RNA-based nature.

However, mRNA is a molecule that is easily degraded due to its short half-life and its sensitivity to RNases. For this reason, it is essential to optimize the vaccine by incorporating delivery systems that ensure the molecule reaches the intracellular environment. These may include LNPs, the most widely used system currently, responsible for mRNA delivery in SARS-CoV vaccines, as well as polymer-based nanoparticles, peptide-based nanoparticles, and virus-like platforms. Moreover, messenger RNA is inherently immunogenic, which can both support the vaccine's intended effect and contribute to its degradation. Therefore, further optimization of the molecule is crucial to advance clinical trials and achieve more advanced stages of vaccine development, with the goal of making cancer vaccines widely available to the population.

In summary, messenger RNA vaccines prove to be a promising approach, with significant potential for growth and substantial benefits as a novel strategy for cancer treatment. As a personalized immunotherapy, they are less likely to cause adverse effects in patients while also being more effective. However, further optimization is still required for these vaccines to become viable for real-world treatment, moving beyond clinical trials which, although promising, still have a long path ahead.

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