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REVIEW BASED BOOK CHAPTER

mRNA SCAFFOLD AND CANCER IMMUNO-THERAPY: BIOCHEMICAL AND CLINICAL ASPECTS

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Abstract

Messenger RNA (mRNA) is in the spotlight as a striking tool for the intervention of various human disorders chiefly malignant tumors, now a days. Thanks to the excellent clinical consequences of mRNA vaccines. Recent propitious preclinical and clinical outcomes that typify the headway in mRNA and nanoformulation-based delivery scaffolds have illuminated the efficacious aptitude of mRNA in cancer immunotherapy. The mRNA can be used for cancer immunotherapy in the form of many therapeutic techniques like adoptive T-cell therapy, therapeutic antibodies, cancer vaccines and immunomodulatory proteins. This chapter highlights the comprehensive overview of the evolving role of mRNA technology against cancer, the underlying mode of action of various types of emerging mRNA-based cancer vaccines as well as immunomodulatory proteins, current status and anticipation of mRNA-based therapeutics inclusive of various delivery strategies as well as has opened new avenues to overcome the deficiencies.

Keywords

mRNA Technology, Cancer Immuno-therapy, Biochemical, Clinical Aspects, Recent Study

1. Introduction

A massive revolution in technology has been grounded over the past decade using mRNA-based technologies, most significantly in cancer therapeutics. Tumor regression was reported in patients injected with samples of erysipelas samples in the 19th century after vaccine trials began but with no significant success [1]. Among many clinically approved vaccines, Sipuleucel-T, a dendritic cell-based vaccine aiming to combat PSA remains the only promising vaccine in the 19th century [2].

To lay the foundation for the development of cancer therapeutic vaccines, the recognition of tumor neoantigens has played a very substantial role. The central pillar of cancer immunotherapy is to stimulate the cytotoxic T cells to abolish cancer cells, which involves directing the body's immune system and inducing immunity against tumors [3]. The adaptive immune response is initiated when T cells identify specific neoantigens of cancerous cells processed by APCs, such as DCs and tissue macrophages. These APCs are responsible for defining the channels of the immune-related cell-killing response [4]. In cancer immunotherapy, mRNA-based vaccines have come up with many new advantages.

The tremendous advantages of mRNA-based vaccines have taken the storm by their feasibility, charge, and production as compared to DNA-based vaccines. mRNA-based vaccines have removed many drawbacks of nucleic acid-based vaccines such as inactivity of DNA integration. Moreover, mRNA-based vaccines can be manufactured in less time, without any comprehensive techniques that have enabled great precision in tumor-directed genomic targeting [5].

Researchers have demonstrated the generation of robust proteins in preclinical studies that have cytotoxic activity [6, 7]. Intracellular machinery is required to synthesize the required antigens by introducing tumor-specific antigens into antigen-presenting cells. Despite the breakthrough in mRNA-based immune-oncology, researchers still need to focus on many challenges, especially the real-life routine practice of these technologies. Moreover, much effort has been made in the field of molecular engineering through the optimization of delivery systems.

As a result, the stability and translational ability of delivery systems have been improved, inherent immunogenicity has been mitigated and intracellular uptake has been increased enabling them in preclinical and clinical studies.

2. The Emerging Role of mRNA Technology in Cancer Immunotherapy

An immune response is generated by nucleic acids whether endogenously or exogenously, both in the form of DNA or RNA. For exogenous immune responses, viruses and bacteria are the main causes, while endogenous response is mainly induced by the shedding of cancerous cells [8]. The cancer-specific immune response is host-specific, and it is generated by introducing the cancer-specific antigens into the host cells to produce the cancer antibodies or structural proteins of the antigens. These cancer antigens may be in the form of nucleic acids, either DNA or RNA, whole cell lysates, or peptides. Collectively, the immune response specific to cancer is otherwise known as the "cancer vaccine".

Due to the efficient delivery systems and full-length tumor antigens, nucleic acid vaccines produce a broader and stronger immune response, as compared to whole-cell or peptide-based vaccines. A good vaccine not only has efficient delivery and safety methods but also can translate into dividing and non-dividing cells. As the mRNA vaccines could not integrate into the host genome. This attribute made them an appealing alternative to DNA vaccines. One of the best examples of in-vitro transcribed RNA vaccines is the SARSCoV-2 cancer vaccine. SARSCoV-2 cancer vaccine. In addition to their versatility, SARSCoV-2 vaccines can encode unique peptide structures, allowing them to pinpoint cancerous cells of different mutational profiles [9].

The processing of synthetic mRNA into the host cell occurs through a series of intracellular events. Firstly, the mRNA penetrates the host cell either via the cell membrane or cytosol. The endocytosed mRNA, which is encoded antigen, is translated into a specific peptide protein by ribosomes. The peptides undergo PTMs that ultimately result in the degradation of peptides within intracellular compartments, making them indistinguishable from the endogenous mRNA product [10]. The encoded antigen thus undergoes two phenomena: followed by either CD4 (+) T lymphocytes or CD8 (+) cytotoxic T cells. To

activate the CD4 (+) T lymphocytes, the encoded antigen enters the autophagic pathway through the process of endocytosis. Thus, the antigens are degraded into epitomes or lysosomal fragments by lysosomes. These epitomes finally join forces with the MHC-II complex located at the surface of the RER and move toward the cell membrane to activate the CD4 (+) T lymphocytes.

On the other hand, CD8 (+) cytotoxic T cells are activated when the encoded antigens are broken down into epitomes or small peptide fragments with the help of proteasomes by proteasome-mediated degradation. The protosomal fragments present themselves to the CD8 (+) cytotoxic T cells after combining with the MHC-I complex at the surface of the rough endoplasmic reticulum.

Endogenously mRNA produces a cancer-specific immune response, while exogenously it triggers the secretion of Type-1 Interferons (IFN) and many other inflammatory cytokines to maintain an immune-friendly microenvironment (TME). These inflammatory cytokines are released by the activation of retinoic acid-inducible gene I (RIG-I) and toll-like receptors (TLR) [11].

There are many advantages of mRNA constructs. They have a significant role in genetic engineering to produce varieties of interleukins: IL-15, IL-12, IL-7, and IL-2, and many kinds of pro-inflammatory cytokines. These cytokines and interleukins create a long-lasting immune response by inducing CD8+ helper T lymphocytes or memory T-cells. The expression of mRNA enhances the production of antigen-specific CD 8 + cytotoxic T cells and reduces the proportion of immune suppressor Tregs to active CD8 cells [12, 13].

Another key role of mRNA construct is in passive targeted immunotherapy where they are used to induce monoclonal antibodies and produce stable antitumor effects. These specific monoclonal antibodies are used to treat various kinds of lymphomas and breast cancer like Her-2 positive breast cancer by inducing a more robust immune-mediated elimination of tumor cells [14, 15] [16]. The endoplasmic events for processing of mRNA by antigen-presenting cells have been presented in Figure 1 [17].

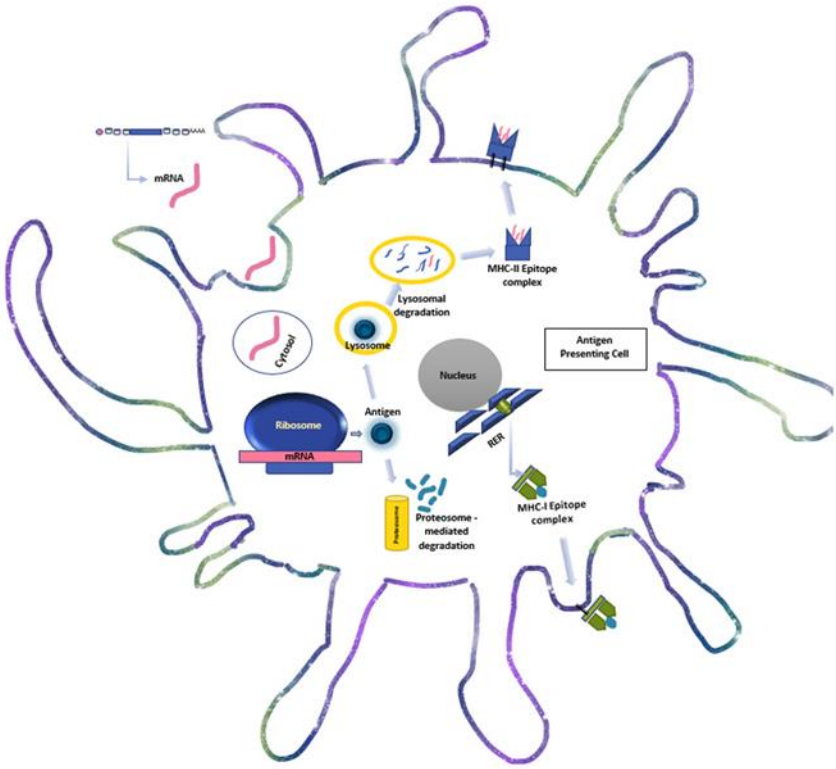

Figure 1 Illustrate the endoplasmic events for processing of mRNA by antigen presenting cells. First, mRNA entry is made into cell through cytosol and corresponding antigen is made by ribosomes through translation. This antigen obeys two main pathways 1. Either antigen degradation is done by the proteosomes leading to formation of small fragments and epitopes, before being transported to the cell membrane they combine with MHC-I complex located on RER and presented as CD8 (+) receptors. 2. Or come into autophagic pathway, through endocytosis to re-enter the APCs. After that degradation is done by the lysosomes to break antigen into epitopes and small fragments ensuring their binding to MHC-II complex and exposed as CD4 (+) receptors.

3. mRNA Molecules for Therapeutics

In studies involving the delivery of mRNA into tissues and cells, IVT (in vitro transcribed) mRNA is commonly used. This IVT messenger RNA is created to imitate natural mRNA, comprising a single-stranded structure containing essential parts including a coding sequence edged at both ends by untranslated regions (UTRs), a 3'-polyadenylated tail, and a 5' cap. Alterations to IVT mRNA have a significant role in increasing its stability and decreasing its capability to stimulate an immune reaction. In 5' capping, the natural m7G

is substituted for an anti-reverse cap analog to avoid reverse incorporation and boost translational efficiency [18]. Modification of the poly(A) tail involves deleting the overhang region at its 3' end during the process of in vitro transcription, forming an "unmasked" polyadenylated tail [19]. The selection of 3' and 5' UTRs is important too, with UTRs derived from genes having high expression, for example, β- or α-globin [20]. The coding sequence exhibits flexibility to some degree, enabling optimization by substituting rare codons for ones occurring more frequently and optimizing the context of codons for improved protein expression [21]. To handle potential immunogenicity challenges, in-vitro transcribed messenger RNA can be purified through high-performance liquid chromatography, which effectively removes double-stranded RNA [22]. Chemical modifications, for example, 2-thiouridine, pseudouridine, and remaining, are incorporated for de-immunization of IVT mRNA [23, 24]. Regardless of these alterations, employing naked altered mRNA for both transfection and treatment remains comparatively unproductive in vitro as well as in vivo [25, 26]. To rectify this, molecules of mRNA are packed inside nanoparticles, which substantially boost cellular uptake and secure them from RNase damage. Nanoparticles may also assist in lysosomal escape and ensure an adequate amount of mRNA to translate. Moreover, a few complexes or delivery vectors created upon formulating with mRNA exhibit adequate adjuvant effects, which makes them significant for the development of mRNA-based vaccines. In this outline, nanoplatforms are required to enhance the mRNA-based therapeutics' potential [27, 28].

4. Structure of mRNA Vaccine

mRNA vaccine is composed of three basic parts: an open reading frame, 3' and 5' non-coding regions, a universal 5' cap, and a 3' poly A tail. The preserved mRNA 5' cap is present in all eukaryotes, and it consists of m7GpppN. The basic function of a 5' cap is to protect the quick degradation of mRNA. Moreover, the 5' cap also induces the IFN-mediated immune responses. The coding regions consist of codons that code for a specific gene of interest. On the opposite side, mRNA's non-coding or untranslated regions enhance the translational efficacy of mRNA. To maintain the stability of mRNA, the poly-A tail plays an important role. In the market, two kinds of mRNA vaccines are

convenient, one is self-replicating (SRM) and the second one is a non-replicating mRNA vaccine. The schematic diagram of non-replicating and self-replicating mRNA vaccines is given in Figure 2 [29-32].

The non-replicating mRNA vaccine has some disadvantages as compared to the self-replicating mRNA vaccine. NRM is less stable and less active as compared to SRM. Due to this reason, there is less demand for NRM, but now up to a certain extent they can be optimized structurally. SRM vaccines are more efficient in their working as they are produced by the genetic engineering of viral structural genes of single-stranded RNA. The viral structural gene in the coding region is substituted by the gene of interest, for example, cancer antigen. SRM maintains a high level of gene expression as the non-structural genes do not degrade and remain intact. Alphaviruses, picornaviruses, and flaviviruses are some of the most common examples of SRM vaccines [6, 33-36].

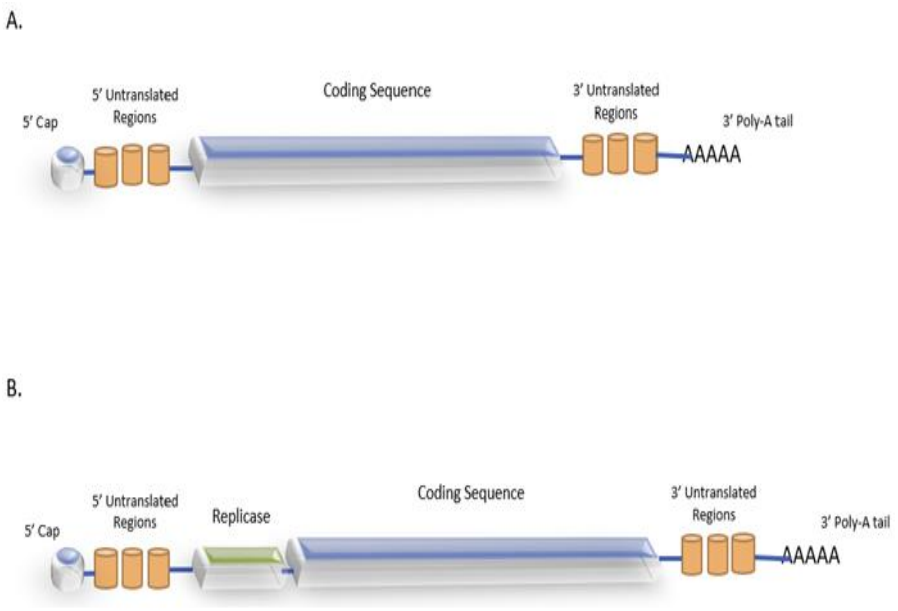

Figure 2 Shows blueprint for the manufacturing of mRNA vaccine 1. Non replicating version 2. Self-replication, during the evolution process all eukaryotic mRNA have 5′ cap that featured the m7GpppN structure, due to its dual nature, it helps the mRNA from degradation as well as facilitate the binding of mRNA with eIF that helps in activation of translation. Codons in coding regions have genes of interest, and anticodon regions modulate the productivity of translation. While poly A tail appears to preserve the integrity of R [12-15].

5. The Underlying Mechanism of mRNA Cancer Vaccines

Most cancer vaccines that are mRNA-based when administrated at the injection site are taken up by the tumor surveillance cells which are competent DCs and APCs of the immune system [37, 38]. Furthermore, cytoplasmic ribosomes acted on internalized mRNAs to form antigenic proteins by translation later followed by ubiquitination, a type of post-translational modification. Subsequently, the Protease complex degrades the antigenic proteins into their respective antigen fragments, and these antigens are presented by two major pathways through the major histocompatibility complex (MHC) class I. Through the transporter associated with antigen processing (TAP) protein, fragments of resulting proteins are transported to the RER and ultimately bind to MHC class I and are displayed at the cell membrane surface for recognition. Activated CD8+ T cells after identifying antigens, secrete cytokines that initiate apoptosis in cancer cells such as granzyme and perforin [39, 40]. The further immune response of anti-cancerous cells can be enhanced by the association of mRNA-containing pathogen-associated molecular patterns (PAMPs) and intracellular pattern recognition receptors (PRRs) [41]. In particular, during endocytosis exogenetic mRNA is identified by the Toll-like receptors (TLRs) of endosomes such as TLR8 and TLR7. Successively, to initiate signaling pathways TLRs recruit adaptor proteins, such as MyD88 and TRIF, which help in activation of transcription factors NF-jB, IRFs, or MAP kinases. Such pathways initiate the release of type I interferon (IFN) and pro-inflammatory cytokines which collectively activate strong adaptive immune responses such as improving T cell immunity against the cancerous cells [42, 43].

Nevertheless, the Stimulation of the overreacted immune system by type I IFN causes to stop the translation of mRNA and even degrades the molecule, and this can be further intensified by dsRNA a product formed during In-vitro transcription for the synthesis of mRNA. Further studies showed that dsRNAs can activate the multiple intracellular pattern recognition receptors PRRs. These PRRs are oligoadenylate synthetase (OAS), endosomal TLR3, RNA-dependent protein kinase (PKR), and cytoplasmic melanoma differentiation-associated-5 (MDA-5). For instance, 40–45 bp-sized dsRNA is recognized by the TLR3 that further amplifies the type I IFN response [44]. On the other hand, RIG-1 and MDA-5 can

bind with 50- triphosphate short dsRNA and approximately size of 2 kb with dsRNA respectively, resulting in activation of surplus gene that can impede mRNA translation [45-48].

To preserve the intracellular effectiveness of vaccines many researchers have explored different strategies aimed at mitigating this excessive immune response. Moreover, despite adding modified nucleosides to thwart immune response by dsRNA, scientists also improved the integrity of IVT mRNA [49]. dsRNA removal helps in achieving high-purity IVT mRNA by using cellulose purification techniques or HPLC [22, 50]. Summary of mechanism of action of mRNA-based vaccine is showed in Figure 3 [51].

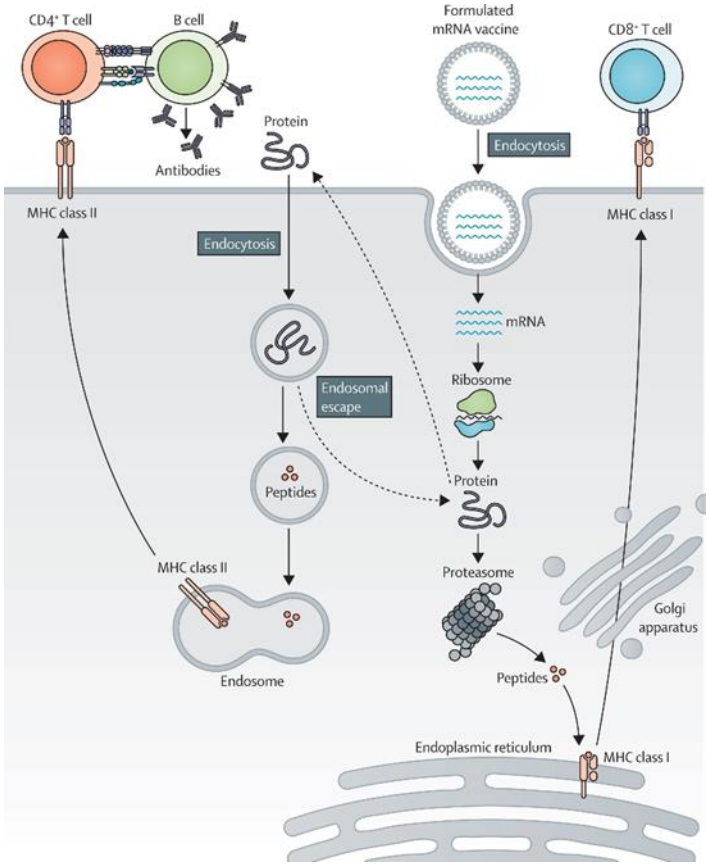

Figure 3 Working principle of mRNA-based vaccine. Activation of antigen-specific CD8+ T-cell followed by the uptake of mRNA by antigen-presenting cells leading to laden on MHC class I. For activation of CD4+ T-cell cross- presentation of extracellular protein on MHC class I or loading on MHC class II is done. CD4+ T-cells could co-stimulate protein-specific B cells, in turn, B cells capable of activating CD4+ T cells followed by the internalization of receptor-mediated antigen.

6. DC Vaccines Using Ex Vivo mRNA Delivery

The pioneering concept, of mRNA-based DC vaccine, played a significant role in the realm of cancer immunotherapy. Regardless of some limitations of mRNA-loaded DC vaccine such as less optimal clinical outcomes and restricted T-cell responses [52], various research studies have shown their ability to delay or even prevent the reappearance of disease, subsequently extending the overall survival rate [53, 54]. A complicated and costly manufacturing process is required for the immediate rescue of mRNA vaccine into isolated dendritic cells such as 1) Withdrawal of DC from the peripheral blood of the patient [55]. 2) The induction and maturation of DCs are done with the association of interleukin-4 (IL-4) and granulocyte–macrophage colony-stimulating factor (GM-CSF) along with using electroporation, delivery of antigen-coding mRNAs into DCs is done [56]. 3) mRNA-based DC cancer vaccine administrated back through subcutaneous, intravenous, and intranodal injections. Clinical trials of phase I and I/II, mRNA-derived DC vaccine provide a huge safety profile in different types of carcinomas. These include patients with androgen-resistant prostate cancers, pediatric neuroblastoma, adult brain cancers, pediatric brain cancers, and renal cell carcinoma [10]. Furthermore, most of the ongoing and completed human clinical trials showed positive results. For instance, a clinical trial of DCs (NCT02808364) pickled with the modified TAAs mRNAs, incorporation with the anti-PD-1 anti-body (nivolumab), yielded promising results with TAA-specific T cell response in patients with advanced lung cancer and glioblastoma multiforme (GBM) without showing any serious effects [57]. The clinical trial of phase II (NCT00965224) deals with acute myeloid leukemia patients in remission with mRNA-based DC vaccines based on Wilms' tumor 1 (WT1). This approach will help in the prevention and delay of disease along with a five-year survival rate in 43% of the vaccinated patients [9, 58]. Additionally, during the clinical study of phase III, metastatic carcinoma of the renal cell-diagnosed patient is treated with DCs loaded with CD40L-encoded mRNA and amplified tumor RNA combined with tyrosine kinase inhibitors such as sunitinib. Tumor regression is achieved by sunitinib due to its ability to inhibit VEGF activity and its non-specific immunostimulant effect, which includes scaling down Treg cells [59]. However, this combination therapy has no significant effect on patient survival [60]. In an investigation in a phase II trial, scientists found that TAA mRNAs-based Trimix DC vaccines given along with anti-CTLA-4

antibodies such as ipilimumab, will help in an increased level of CD62Lhigh Tregs in peripheral blood and a response rate is 38% partial or complete [53]. Currently, three scientific trials are ongoing for the assessment of mRNAs-based DC vaccines encoding antigens in combination with anti-CD27 antibodies (NCT04911621, NCT04911621, NCT03548571) and chemotherapy in glioblastoma patients. The overview of the analysis of proceeding clinical examinations for mRNA-based cancer vaccines has been presented in Table 1.

Table 1 Overview of clinical trials for mRNA-based cancer vaccines

Therapeutic approach	Name	Antigen/ Cargo	Co-treatment	Patient number	Tumor type	Injection site	Phase	NCT number	Status
Formulated mRNA	mRNA-2752	OX40L, IL-36c, IL-23	Durvalumab	264	Lymphoma, or Solid tumors	i.t	I	NCT03739931	Recruiting
	BNT111	NY-ESO-1, tyrosinase, TPTE, MAGE-A3,	Cemiplimab	180	Melanoma	i.v.	II	NCT04526899	Recruiting
	W_ova1	Ova	-	10	-	i.v.	I	NCT04163094	Active
	BNT112	undisclosed antigens, PSA, PAP3	Cemiplimab	115	Ovarian cancer Prostate cancer	i.v.	I/II	NCT04382898	Active
	BNT113	HPV16-derived tumor antigens E7, E6	Pembrolizumab	285	HNSC	i.v.	II	NCT04534205	Recruiting
	BNT116	Commonly expressed by NSCLC	Docetaxel, Cemiplimab,	80	NSCLC	i.v.	I	NCT05142189	Recruiting
	mRNA-4157	Patient-specific neoantigens (up to	Pembrolizumab	157	Melanoma	i.m.	II	NCT03897881	Active

		34)							
	MEDI1191	IL-12	Durvalumab	102	Solid tumors	i.t.	I	NCT03946800	Recruiting
	BNT122	20 patient-specific neoantigens	Pembrolizumab	131	Melanoma	i.v.	II	NCT03815058	Active
Naked mRNA	BNT151	IL-12	–	84	Solid tumors	i.v.	I/ II	NCT04455620	Recruiting
	SAR441000	IFN-a, GM-CSF, IL-15, IL-12,	Cemiplimab	77	Metastatic Neoplasm	i.t.	I	NCT03871348	Active
	Trimix	IFN-a, GM-CSF, IL-15, IL-12, CD40L, TLR4, CD70,	–	36	Breast cancer	i.t.	I	NCT03788083	Recruiting
	BNT153, BNT152	IL-12, IL-7	–	112	Solid tumors	i.v.	I	NCT04710043	Recruiting
mRNA-transfected DC	–	pp65-LAMP	Varlilumab, Temozolomide	80	Glioblastoma	i.d.	II	NCT03688178	Recruiting
	–	Survivin, hTERT,	Temozolomide	60	Glioblastoma	i.d.	II/ III	NCT03548571	Active
	–	WT1	Temozolomide	10	Glioma	i.d.	I/ II	NCT04911621	Recruiting

7. In Vivo mRNA Cancer Vaccines

In vivo, cancer vaccines that are mRNA-based represent a separate type of mRNA vaccine currently being trailed in clinical trials because of the few advantages they possess as compared to mRNA-based DC vaccines, like their comparatively lower cost, smooth production process (having fewer irregularities between batches), and simpler scalability [6]. This method requires direct delivery of mRNA-based vaccines, packed within a delivery carrier or not, into individuals suffering from different cancers, which include melanoma (NCT03394937), gastrointestinal cancer (NCT03480152), lung cancer of the non-small-cell type (NCT00923312), and prostate cancer (NCT01817738). An active

clinical trial is assessing the productiveness of a liposomal RNA-based vaccine (RNA-LPX), FixVac (BNT111), that aims at targeting four unmutated TAAs that are commonly found in melanoma. Within this investigation, individuals suffering from advanced melanoma were administered an intravenous dose of RNA-LPX either solely or alongside an anti-PD-1 antibody. Positively, durable concrete outcomes have been noticed (NCT02410733) [61]. Additionally, mRNA-2752, which is a combination of 3 mRNA species that encode IL-36 gamma, OX40L, and IL-23, is presently being evaluated as part of a dose-escalation study within patients suffering from lymphomas (NCT03739931) and solid tumors, depending on preclinical outcomes in which the formulated vaccine's intratumorally infusion caused existing tumors' regression within 3 syngeneic tumor models [62].

8. mRNA-based Therapeutic Protein Production

Replacement therapy for Protein serves to compensate for a shortage in a certain protein that may be missing or defective because of genetic mutations within the diseased individual. On the other hand, gene-based therapy has been thoroughly researched to assist with the formation of active proteins within patients, which results in the current approval from the FDA for gene-based therapy of hemophilia (Hemgenix) [63]. Within cancer therapy, mRNA could be employed to bring immunomodulatory or immunotherapeutic agents, for example, immunostimulatory cytokines and anti-cancer antibodies. While utilizing mRNA to generate immunomodulators within the living body, a significant rate of protein formation is needed to attain therapeutic concentrations effectively, potentially administrating of multiple dosing [64]. Moreover, the administration of systemic mRNA-LNP complexes leads to their substantial unintended piling up within the liver. That is why attaining ideal therapeutic effectiveness requires the design of an effective delivery strategy aimed at specific sites of tissues. Notably, comprehensive investigations are in progress to find the best lipid ratios and compositional modifications within LNPs [65]. Even though protein production methods based on mRNA involve multiple challenges like delivery to intended sites as well as high production efficacy, they are presently being based ponied within many clinical trials for multiple cancers like melanoma, ovarian cancer, breast cancer, and lymphoma. In light of the diverse and changing nature of different cancers, this generally suitable method,

irrespective of the particular cancer kind, has been of immense importance in further promoting an anti-cancer immune reaction solely or alongside other immunotherapies [66].

9. mRNA-encoded Immunomodulatory Proteins

Various immunomodulatory proteins like cytokines, chemokines, and TLRs, as well as co-stimulatory ligands, modify the immunological microenvironment of the tumor and promote successful anti-tumor immune responses [67]. However, given their dose-linked toxic effects and short half-lives, the immunomodulators' systemic delivery has several limitations in the clinical setting. mRNAs inside delivery carriers are the most appropriate treatments for resolving these issues; the productivity of mRNAs encoding multiple co-stimulatory ligands and cytokines has been examined through clinical tests.

9.1. Cytokines

IL-12 controls TH1 immunity and supports efficient anticancer effects within mouse tumor models; in contrast, it is fatally harmful when given systematically to humans [68]. For this reason, an IL-12 messenger RNA agent (MEDI1191) was provided intratumorally coupled with an anti-PD-L1 antibody (durvalumab) to patients suffering from late-stage solid tumors (NCT03946800). The findings of this clinical study demonstrated that the use of MEDI1191 alongside durvalumab was easily tolerated, led to a transient rise in IL-12 serum levels, and also showed tumor shrinkage [69]. In separate research, Hotz et al. explored the anti-tumor impact of intratumor administration of mRNAs encoding 4 cytokines that cause tumor shrinkage (IFN-a, IL-12 single chain, IL-15, and GMCSF). Administration of the saline solution comprising the mRNA mixture led to intratumorally IFN-gamma induction, expansion and infiltration of antigen-targeted T cells and immunological memory development within various tumor models. Therefore, a clinical study of the mixture of mRNAs encoding cytokines in individuals suffering from late-stage solid tumors was begun (NCT03871348) [70].

9.2. Co-stimulatory Ligands

OX40L, which interacts with OX40 to boost the expansion of CD8+ and CD4+ T cells and the development of memory T cells and suppresses the function of Treg cells, was formulated for therapeutic usage as an mRNA (mRNA-2416) and underwent its initial clinical trial for individuals suffering from solid tumors (NCT03323398). Within this clinical investigation, intratumorally dosing of mRNA-2416 solely or alongside durvalumab enhanced expression of the OX40L protein and increased pro-inflammatory action [71]. Likewise, mRNA-2752 encoding OX40L, IL-36 gamma, and IL-23 pro-inflammatory cytokines were explored in a first human trial of 23 patients with solid tumors as monotherapy or alongside durvalumab (NCT03739931). The delivery of mRNA-2752 solely and alongside durvalumab was easily tolerated at every dose level examined, and healing was related to tumor shrinkage [72]. Trimix is a mixture of mRNAs encoding immunostimulatory proteins or antigens, including TLR4, CD40L, and CD70. Administration of Trimix mRNA in tumor-bearing mice resulted in the maturation of CD8a+ DCs associated with the tumor and stimulation of T cells, leading to slowed tumor growth [73]. Clinical investigations are presently being conducted to verify the safety and immunoregulatory response of intratumoral administration of TriMix in patients suffering from initial-phase breast cancer (NCT03788083) [74].

10. mRNA Delivery Platforms

The delivery of mRNA into the host cell is carried through many routes such as intramuscular, subcutaneous, and intradermal. For an effective immune response in the field of mRNA technology, researchers have to face many challenges like rapid degradation, short half-life, inadequate immune response, etc. Due to the large size and negative charge of RNA, it faces some difficulty in passing the intracellular cell membrane. Once mRNA surpasses the significant barrier, it has to face another significant problem. The large number of ribonucleases throughout the systemic circulation and skin cells causes the huge degradation of mRNA. Therefore, it is vital to deliver mRNA efficiently into the cell if we are to achieve the desired therapeutic effects. Nanotechnology has been developed to deliver therapeutic mRNAs in a manner that ensures optimal translational efficiency [6, 9, 75].

10.1. Lipid-based Delivery System

To achieve the best possible therapeutic results of mRNA, lipid-based delivery systems have been considered the fastest and most extensive delivery systems. In lipid-based delivery systems, lipid nanoparticles are the basic structure that consists of many components such as cholesterol, cationic or positively charged ionizable lipid layer (pH-dependent), phospholipids, and polyethylene glycol (PEG) [75, 76].

All these components perform different functions. The function of polyethylene glycol or PEG is to prevent the lysosomal degradation of mRNA. Cholesterol provides structural stability to the mRNA molecule [77, 78]. Efficient delivery, tissue degradation, and tolerance are the key functions of phospholipids [79]. Lastly, the negatively charged mRNA molecules remain encapsulated within the ionizable amino lipid layer that facilitates endosomal uptake of the liposome too [77, 78, 80]. The summary of the effects of lipid nanoparticle vaccines is represented in Figure 4 [81].

Several clinical studies have been done to optimize the efficient delivery, long-lasting, and strong humoral immune response of LNP mRNA vaccines in different cell lines such as murine models [82-84]. Several clinical and Preclinical studies in LNP mRNA vaccines also provide antigen-specific antitumor activity in memory T cells while preventing the growth of tumors in many murine models. LNP or Lipid nanoparticle technology delivers the cancer vaccine specifically to antigen-presenting cells rather than retaining effective translational efficiency and preventing degradation of mRNA [85-88].

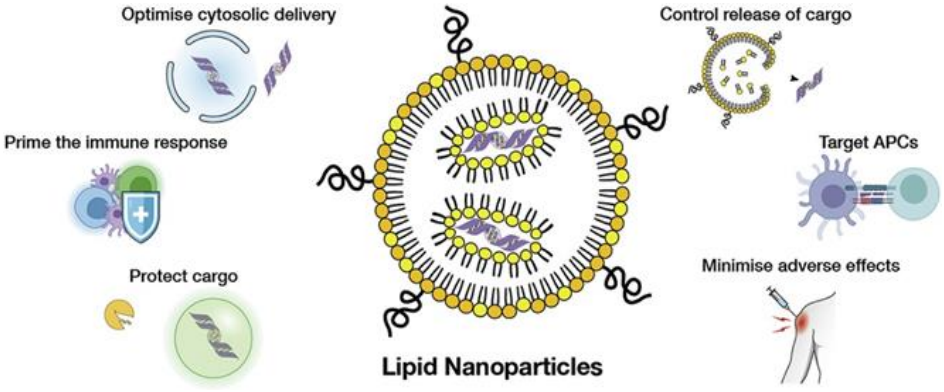

Figure 4 The summary of effects of lipid nanoparticles vaccines

10.2. Delivery Systems Utilizing Polymers

Dendrimers and nanotechnology-modified polymers have become popular for delivering mRNA for vaccines that target deadly viral pathogens such as Ebola, influenza viruses, HIV, H1N1, and Zika [89-92]. The translation of similar mRNA vaccines into tumor-associated antigens can also be demonstrated in vivo [93]. Polymeric structures covered with PEG outer shells have been used to deliver an antiangiogenic RNA sequence to murine models, which inhibited pancreatic cancer growth [94]. Furthermore, castration-resistant prostate cancer models have been successfully treated with polymer-based RNA vaccines encoding PTEN, and polymeric-based RNA vaccines have been shown to inhibit the growth of tumors [95]. The summary of the mechanism of action of polymer-based vaccine is represented in Figure 5 [96].

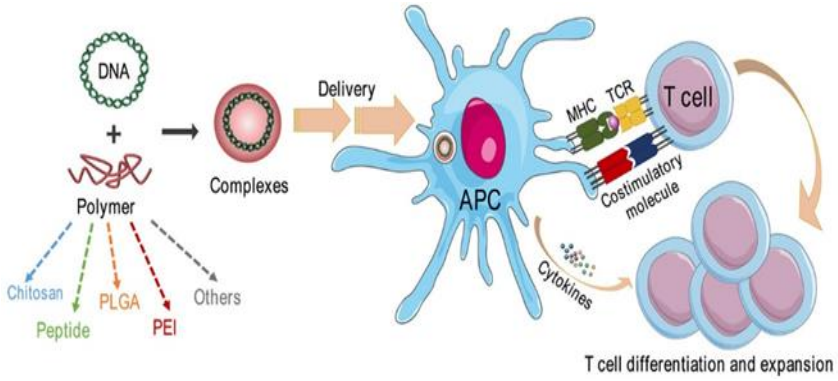

Figure 5 The summary of mechanism of action of polymer-based vaccine

10.3. Peptide-based Delivery

Peptide-based delivery of mRNA consists of cationic peptides that are responsible for the delivery of nanoparticles, oligonucleotides, small proteins, and small organic molecules through cell membranes. This kind of translocation does not need any membrane receptors making Cell penetrating peptides (CPP) a very efficient class of non-viral delivery of mRNA. But still, there are some clinical limitations of CPP such as impaired internalization and low tissue and cell selectivity [97-100].

One of the best examples of peptide-based delivery is protamine-based delivery. The cationic peptide protamine prevents the lysosomal degradation of mRNA and produces

a strong immune response [101]. For example, cationic pegylated KL4 peptide complexes have been used successfully as aerosolized delivery systems for pulmonary administration [102]. The mode of action of the peptide cancer vaccine is represented in Figure 6 [103].

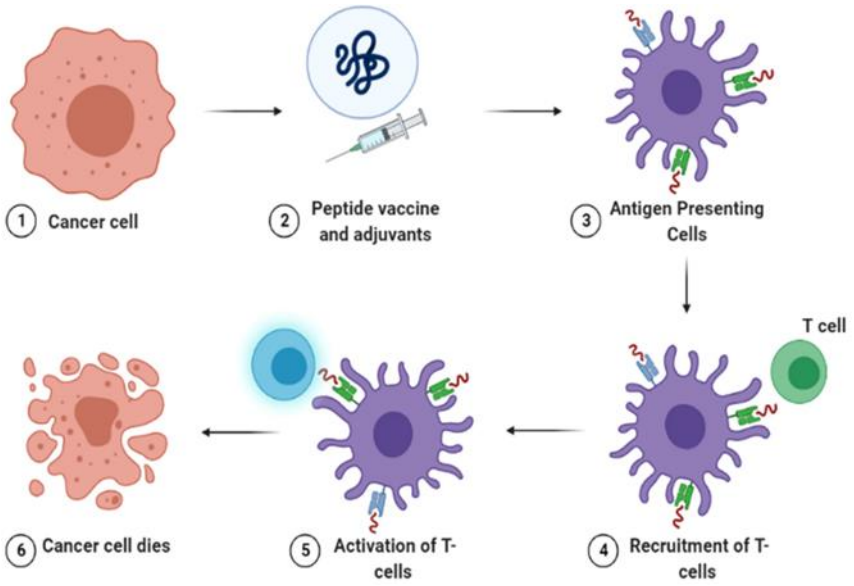

Figure 6 The mode of action of peptide cancer vaccine

The summary of mRNA delivery approaches in cancer therapy is given in Table 2.

Table 2 Summary of mRNA delivery approaches in cancer therapy

Delivery system	Components	Cancer type	Targeting moiety / Site	Injection route	Gene	Ref
LNP	Cholesterol, 113-O12B, DMG-PEG DOPC	OVA mRNA	Lymph node/ 113-O12B	Subcutaneous	B16F10-melanoma	[104]
	L8, DMG-PEG, DSPE-PEG Cholesterol, DSPC	Human OV8 peritoneal xenograft	OV8 cancer Cell / Anti-EGFR	Intraperitoneal	sgRNA, Cas9 mRNA, PLK1	[105]
	Cholesterol, DSPC DOPE, Dlin-MC3-DMA	B16F10 melanoma	None	Intratumoral	OX40L mRNA, si-PD-L1	[106]
	Dlin-MC3-DMA, Cholesterol, DSPC, Ceramide-PEG, Cholesterol	E077-1 tumor model, NDL	circulating and splenic T cells/ DSPE-PEG5K-aCD3	Intravenous	mCherry, Fluc mRNA	[107]

	Cholesterol, Ionizable lipid, PEG-lipid, DSPC	metastasis model, TC-1 Lung, TC-1 tumor model,	None	Intramuscular	STINGV^{155M} mRNA, Antigen (HPV-E6/E7) mRNA,	[108]
Hybrid	mPEG-PLGA, G0-C14	B16F10 Melanoma, Pten-null prostate cancer	None	Intravenous	PTEN mRNA	[109]
Peptide-Based NP	Stearyl-Arg8, RALA, p5RHH	Ovarian cancer xenograft mode	None	N/A	OVA mRNA, mCherry	[110]
	PF14	CL25. CT26. colorectal cancer cell	None	Intraperitoneal	EGFP mRNA	[111]
Polymeric NP	anti-CD8 antibody coupled PGA, PBAE-447	hepatitis B-induced hepatocellular carcinoma, Leukemia, prostate cancer model	Anti-CD8 / circulating T cell	Intravenous	CAR mRNA, TCR	[112]
	GO, LPEI	B16-OVA melanoma	None	Subcutaneous	OVA mRNA	[113]
	CARTs	CT26 and A20 tumor model	None	Intratumorally	CD86, CD80 mRNA, OX40L	[114]

11. Conclusion

mRNA-based cancer immunotherapy has evolved as a promising technique for intervention of cancer. Recent clinical examinations have shown the efficacy and safety of mRNA-based cancer immunotherapy with the achievement of therapeutically significant tumor remission. The durability of mRNA exists in its wide-ranging application window in various modalities of cancer immunotherapy. The mRNA can be harnessed to encode entirely all competitors of anti-cancer immune response inclusive of therapeutic antibodies, antigen receptors, cancer antigens and immunomodulatory cytokines thus enabling the manufacturing of personalized or sophisticated therapies against individual cancers. It is noteworthy that the impact of mRNA is short-lived due to its transient nature

and easily governable and therefore can reduce the risk of long-term toxicity and inaccurate effects. The revolutions of chemical modification enriched mRNA with enhanced translational efficiency to increase the manufacturing potency of encoded proteins. Contrary to viral vectors, LNP has a deficit in immunogenicity and therefore can be utilized again and again for mRNA-based cancer therapy if required for maintained therapeutic efficacy.

12. Future Directions

All in all, clinical studies on cancer immunotherapy-based mRNA have introduced advanced directions for increasing the care of cancer patients. However, there are still awaiting tasks to fully harness the advanced therapeutic strategies including optimization of neo-antigen specific cancer vaccines, managing, and orchestrating the anti-cancer immune response, and apprehension of the biology of immune flee in cancer. mRNA-based cancer immunotherapy is still a novel and rapidly emerging field. Clinical trials, continuous research, and collaborations between clinicians and scientists are important for the advancement of this innovative approach to the treatment of cancer.

Author Contributions

Conceptualization, design, data analysis, results interpretation, editing and final approval, H.S; Writing, editing, original draft preparation, S.S; Writing and data analysis, H.K, F.M, K.T, I.A, A.L, F.F and H.B; Validation, visualization and final approval, Z.K.

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Conflicts of Interest

No conflict of interest is to be declared by any author.

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