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mRNA vaccines in the context of cancer treatment: from concept to application

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Abstract

Immuno-oncology has witnessed remarkable advancements in the past decade, revolutionizing the landscape of cancer therapeutics in an encouraging manner. Among the diverse immunotherapy strategies, mRNA vaccines have ushered in a new era for the therapeutic management of malignant diseases, primarily due to their impressive impact on the COVID-19 pandemic. In this comprehensive review, we offer a systematic overview of mRNA vaccines, focusing on the optimization of structural design, the crucial role of delivery materials, and the administration route. Additionally, we summarize preclinical studies and clinical trials to provide valuable insights into the current status of mRNA vaccines in cancer treatment. Furthermore, we delve into a systematic discussion on the significant challenges facing the current development of mRNA tumor vaccines. These challenges encompass both intrinsic and external factors that are closely intertwined with the successful application of this innovative approach. To pave the way for a more promising future in cancer treatments, a deeper understanding of immunological mechanisms, an increasing number of high-quality clinical trials, and a well-established manufacturing platform are crucial. Collaborative efforts between scientists, clinicians, and industry engineers are essential to achieving these goals.

Keywords Cancer vaccine, mRNA, Tumor-associated antigen, Neoantigen, Lipid nanoparticle, Immunotherapy

Introduction

Cancer has become a public health issue with increasing concern. In the United States, report showed that about 5370 new occurrences and 1670 deaths each day are expected during 2023 [1]. Although remarkable progress has been achieved to combat the dreaded disease during the last decades, such as the exciting success of immune checkpoint inhibitors (ICI), cancer remains a challenging medical problem affecting millions of people around the world [2, 3]. Therefore, the development of more novel and effective platforms for cancer treatment has become an urgent need.

For centuries, population vaccination has already achieved a tremendous success to target varieties of lethal diseases. As a promising alternative approach, cancer vaccines are designed to stimulate the immune system resulting in the control of tumor growth and the

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destruction of cancer cells. Among these, mRNA-based cancer vaccines demonstrate exceptional advantages in comparison with the other types of vaccines [4, 5]. Although mRNA vaccines have been well known because of the significant role in the COVID-19 pandemic, the attempts in the field of cancer have actually been much earlier.

Various platforms of cancer vaccine have their own characteristics with unique advantages and disadvantages. For mRNA-based cancer vaccine, the most limitations related to its vulnerability to degradation, poor delivery system and modest immunogenicity, which hinder its practical application immensely [6]. Fortunately, the rapid advancement in mRNA therapeutic modalities and nanoformulation-based delivery technologies addressed these challenges to a large extent, leading to the emergence of numerous mRNA vaccination platforms for a wide range of cancer. In contrast, mRNA cancer vaccines have manifested several striking advantages over other vaccines, such as great safety without the risk of integrating into the host genome, high efficiency to elicit humoral and cellular immunity, relatively low production costs, and well tolerance [7, 8]. In view of this, while mRNA cancer vaccines are still in the early stages, swift development in this field indicated these vaccines provided new tools and promising platform for oncotherapy, and more efforts should be perform further.

In the present review, we first make a brief review about the mRNA molecule and in vitro transcript (IVT) technology. Subsequently, a comprehensive overview about mRNA-based cancer vaccines was provided, including the classification and corresponding characteristics, the optimization strategies, common delivery tools and administration routes. We also summarized advances of recent clinical trials completed or ongoing in the field. Finally, some major challenges and future considerations for the application of mRNA cancer vaccines were discussed.

History and basics of mRNA vaccine

After the discovery of mRNA in 1961, many outstanding researches focused on the determination of its structure, function, and metabolism in eukaryotic cells [9–12]. Subsequently, evidence that mRNA could be transfected and expressed successfully in multiple eukaryotic cells pushed our understanding of mRNA forwards greatly [13, 14]. At the meanwhile, the concept of mRNA-base therapy has been proposed because of coding function of mRNA for cellular protein, such as antigens, antigen receptors, tumor suppressors as well as cytokines. More importantly, in the context of a few great breakthroughs IVT technology, the first report that the IVT mRNA was successfully validated for synthesizing proteins in vivo was published in 1990, putting the foundation for the development of mRNA vaccines [15–17].

In 1995, the study of Conry et al. first described mRNA vaccination as potential anti-tumor treatment [18]. Since then, extensive preclinical and clinical studies have been initiated and provided substantial evidence that mRNA vaccination can efficiently induce immune responses and eliminate cancer cells. The basic principle of mRNA as a cancer vaccine is that mRNA can deliver the target transcript encoding immunomodulatory molecules, such as tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs), to the cytoplasm of the host cell, and then express them as the activators of the anti-tumor immunity [19, 20]. However, compared with infectious disease vaccines, which target well-defined antigens for prophylactic vaccination, most tumor-targeting antigens display a high degree of interindividual heterogeneity with limited number and poor characters, raising concerns about the practicality, applicability and efficacy of mRNA cancer vaccines. Besides, other important factors, such as the design of mRNA vaccine, the delivery system as well as administration route, involved in the optimization for its therapeutic application. In general, although mRNA cancer vaccines have advanced quickly, there have been many issues remain to be solved to promote the feasibility and efficacy [21, 22]. To date, the U.S. National Library of Medicine (ClinicalTrials.gov) listed more than 100 clinical trials for mRNA vaccines for different types of cancers including breast, ovarian, prostate, glioblastoma, melanoma, colon, non-small cell lung cancer (NSCLC) and so on [23]. However, most of these trials are only at early stage without authoritative and reliable research results yet. Therefore, the clinical outcome for mRNA cancer vaccines is far from clinical relevance and the clinical translation of cancer vaccines is still under a long way. Representative discoveries and breakthroughs in mRNA cancer vaccines were shown in Fig. 1.

Classification: IVT mRNA and beyond

The structure of IVT mRNAs is much similar to that of naturally occurring mature mRNA [24]. In this context, IVT mRNA can theoretically meet all genetic information requirements to encode and express all kinds of proteins. Currently, IVT mRNAs as cancer vaccines can be classified into two categories generally: non-replicating mRNA (also named as conventional mRNA) and self-amplifying mRNA (also called replicon mRNA, SAM) [25]. The former vaccines encode the relevant antigen and contain 5′ and 3′ untranslated regions (UTRs), while the latter SAM vaccines encode both the antigen of interest and designed viral replication machinery, enabling durable intracellular RNA amplification and high translation efficiency. Because conventional mRNA cannot replicate independently and prone to be degraded after

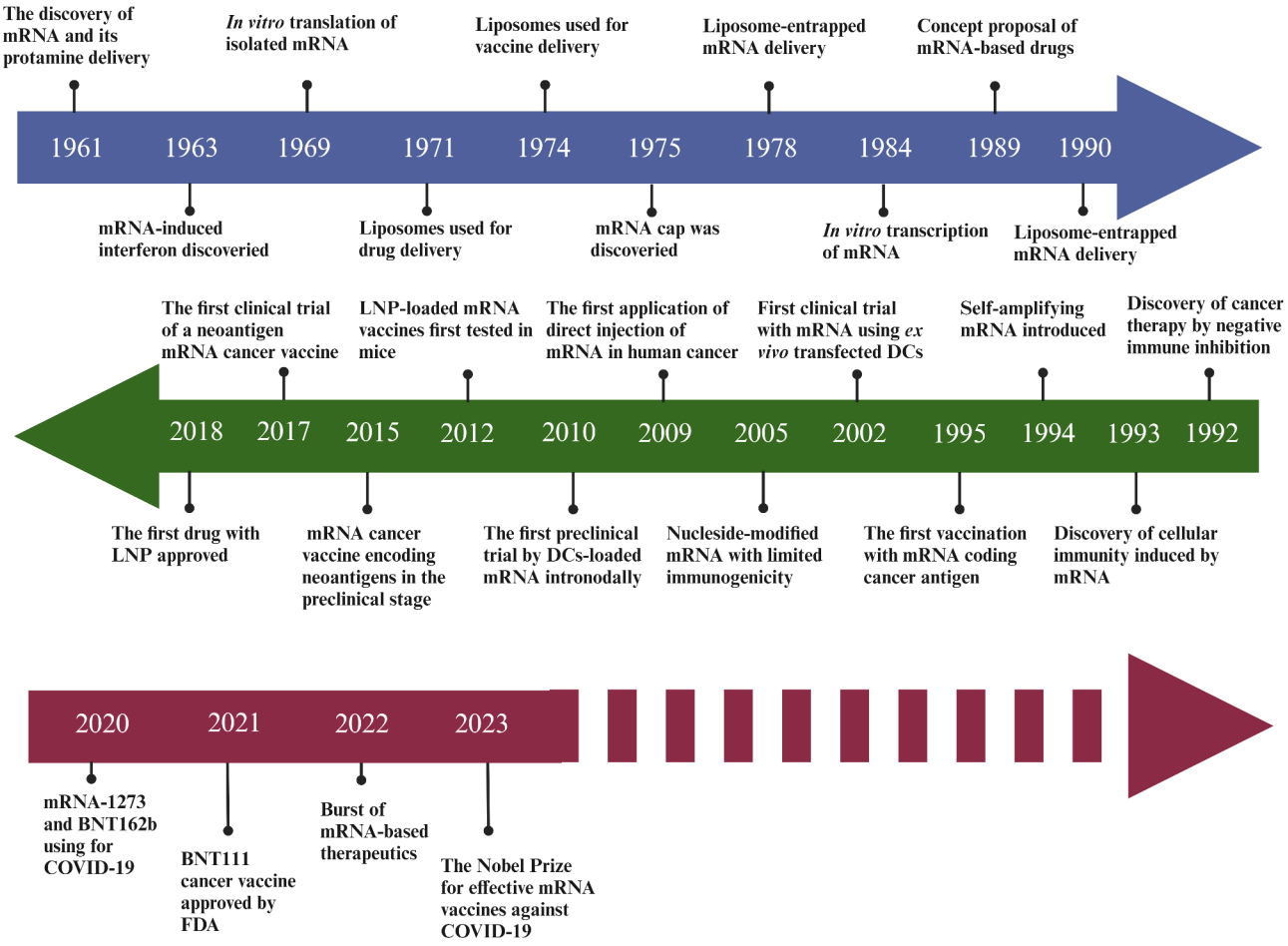

Fig. 1 Timeline of some representative discoveries and breakthroughs in mRNA cancer vaccines. Figure created with BioRender (biorender.com). Abbreviations: DCs, dendritic cells; LNP, lipid nanoparticle; COVID-19, coronavirus disease 2019; FDA, Food and Drug Administration

successfully delivery, the expression of therapeutic protein depends on the number of conventional mRNA transcripts. As a result, repeated dosing of conventional mRNA therapies is required to maintain protein expression, leading to concerns about its long-term safety. In contrast, SAM vaccines have shown effectiveness in generating multiple copies of mRNA in target cells because of its self-replicating characteristics and then prolonged the duration of protein expression (Fig. 2). For instance, it has been reported that regulatory elements of SAM can provide 200,000-fold RNA amplification in transfected host cells. More importantly, SAM exhibit superior immune responses even at 100-1000-fold lower doses compared with immunization with conventional mRNA [26]. Although SAMs provide an appealing alternative for mRNA-based vaccine, to our knowledge, there are only two ongoing clinical trials for SAM vaccines in cancer at present.

In 2020, Beissert et al. made a novel design to the structure of SAM and an improved SAM vaccine termed as trans-amplifying RNA (taRNA) was introduced [27]. This strategy relied on a bipartite vector system composed of two different templates to generate antigen-encoding alphaviral RNA and replicase-encoding RNA separately. The amplification is performed *in trans* by the replicase in the cytoplasm. Compared with SAM, taRNA is much safer and easier to manufacture and functionalize due to the bipartite modality, the shorter length as well as the ease of optimization (Fig. 2). In a mouse model against influenza, taRNA has been used and the results showed that neutralizing antibodies and protective immune responses can be induced at a dose of only 50 nanogram. In addition, circular RNAs (circRNAs) are a class of single-stranded with covalently linked head-to-tail topology, characterized by their robust resistance to exonuclease-mediated degradation and prolonged half-life. circRNAs can be formed through back-splicing during gene transcription, and they can also be chemically synthesized or IVT. The superior stability and high translation efficiency make circRNAs a promising candidate for RNA medicine. For example, the potential use of circRNA-based vaccines in cancer stem cell therapy has been proposed.

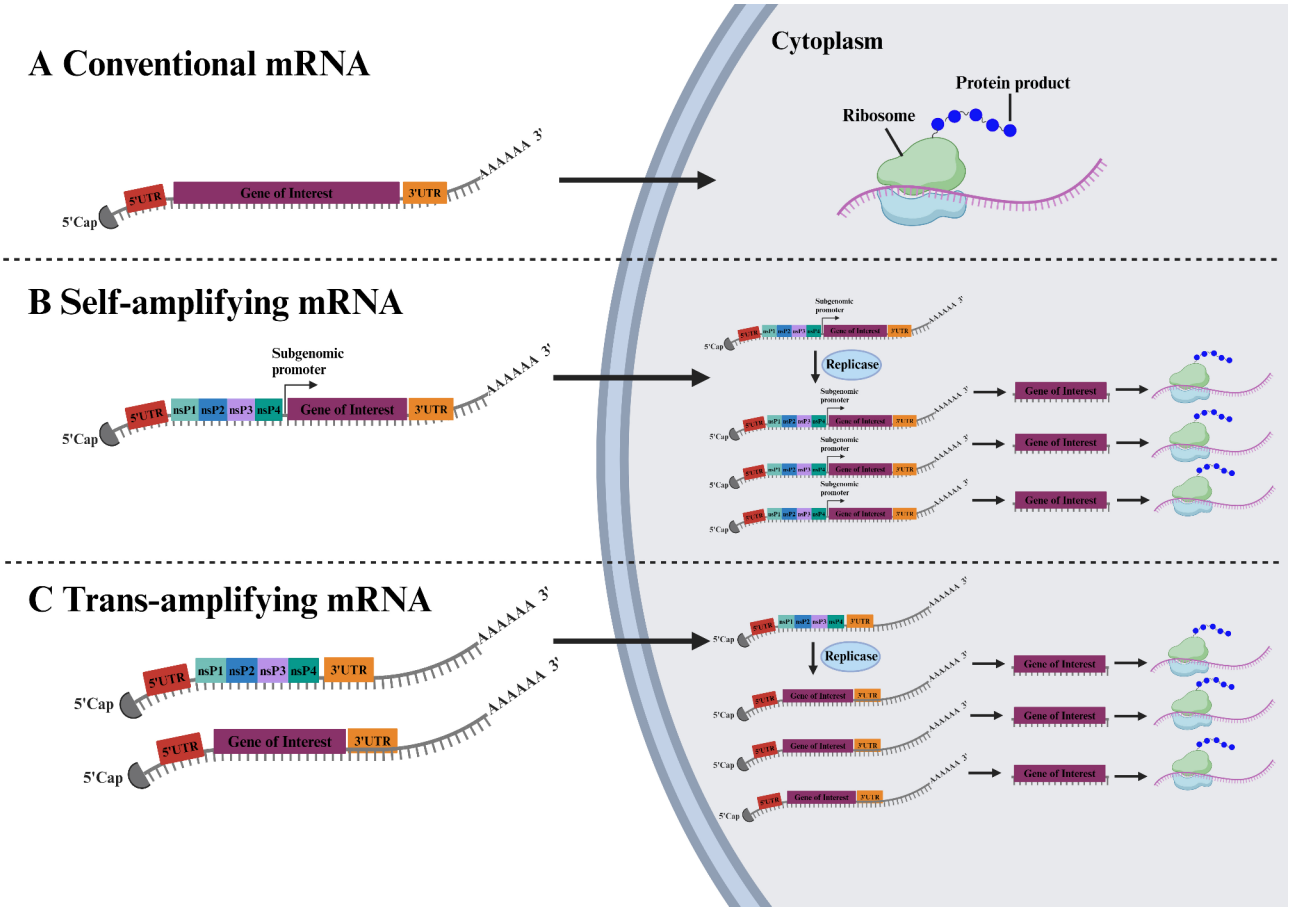

Fig. 2 Schematic diagram of IVT mRNA in cancer vaccine. (**A**) Conventional mRNA. (**B**) Self-amplifying mRNA includes four genes encoding four non-structural proteins (nsP1–4) required for the formation of a functional replicase. Subsequently, the replicase uses the mRNA as a template to self-amplify. The gene of interest can be expressed through the control of a subgenomic promoter. (**C**) *Trans*-amplifying RNA consists of two kinds of mRNAs, one retaining a replicase encoding gene, the other expressing the gene of interest. Figure created with BioRender (biorender.com). Abbreviations: IVT, in vitro transcription; UTR, untranslated region; nsP, non-structural protein

In 2023, Amaya and collaborators performed a systematic evaluation about the adjuvant activity, route of administration, and antigen-specific immunity of circRNA vaccination in mice. Their results highlight the potential utility of circRNA vaccines for stimulating potent innate and T cell responses in tissues [28]. However, investigations of circRNA vaccines are still at the initiation stage, their applications in cancer therapy require further assessment. More recently, chemically synthesized minimal mRNAs (cmRNAs) have emerged as another promising alternative to IVT-mRNA for cancer therapy and immunotherapy. CmRNAs have unique structure without 5'-cap and 3'-polyA tail, which make it more stable and less prone to degradation. A recent study of Yang and colleagues reported that the intratumoral administration of a cmRNA mixture encoding four cytokines elicited a notable tumor-suppressive effect by boosting the infiltration of T cells to facilitate immune therapy. Some research ongoing further demonstrated that the potential efficacy of cmRNA-based vaccines in stimulating robust immune responses and generating long-term immunological memory attributed to various factors, such as efficient translation and antigen presentation pathway. These finding indicate that cmRNA, a pioneering field of mRNA vaccines, may serve as a potential platform in biomedical applications [29]. Collectively, all these IVT mRNAs offer great flexibility as a type of cancer treatment. As data about taRNA-based, circRNA-based and cmRNA-based vaccines is relatively limited, we will focus on the non-replicating mRNA and SAM in the following sections.

Immunogenicity of mRNA vaccine: from paradox to equilibrium

In the development and implementation of mRNA-base cancer vaccine, one of the most challenging and non-negligible issue is about its immunogenicity modulation. Abundant studies suggested that mRNA vaccines have self-adjuvanting properties and the delivery of mRNA vaccines can activate both innate immune response and

adaptive immune response [30, 31]. Immunologically, innate immunity, the first defensive line against non-self substances, can sense mRNA, especially from IVT, and then triggers adaptive immunity through specific signaling cascades. For mRNA-base vaccination, it is required that both innate and adaptive mechanisms work together to induce strong and durable immune responses.

Plenty of studies about mRNA vaccines showed that the response of innate immune can be activated through the recognition of immune cells and non-immune cells, respectively [32]. In immune cells, innate immune can be regulated by various pattern recognition receptors (PRRs) in host immune cells through the detection of pathogen-associated molecular patterns (PAMPs). Therefore, it is essential to understand how diverse cells sense non-self mRNA and initiate cascades of signaling pathways by the interaction of mRNA, PRRs and PAMPs. PRRs of cells can recognize exogenous substances, such as IVT mRNA, resulting in activation of Toll-like receptors (TLRs), particularly highly expressed on antigen-presenting cells (APCs). While TLR3 normally recognizes and binds to double-stranded RNA (dsRNA), recruiting Toll-interleukin-1 receptor-domain containing adaptor inducing-IFN-β (TRIF) to trigger a downstream signaling cascade, single-stranded RNA (ssRNA) is mainly mediated by TLR7 and TLR8, mediated by the myeloid differentiation marker 88 (MYD88)/TLR7-dependent signaling pathway. In non-immune cells, cytoplasmic retinoic acid-inducible gene I like receptor (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) sense exogenous mRNA and regulate the generation of cytokines and chemokines, which results in the induction of anti-viral immunity and the initiation innate immune responds. It should be mentioned that, whatever RNA sensor is, RNA-induced PRRs contribute to type I interferon (IFN I) production increasing the IFN I response and induce a proinflammatory state. However, the increase of IFN I generation and the activation of innate immunity have dual or even paradoxical effects during mRNA inoculation. On the one hand, the complete activation of innate immunity is required for the initiation of adaptive immunity, maintaining mRNA's intracellular activity as a vaccine. On the other hand, excessive innate immunity by IFN I can also result in mRNA degradation and translation inhibition. Therefore, a delicate equilibrium between innate immunity and adaptive immunity should be achieved during the application of mRNA-base vaccines, such as tailoring the mRNA sequence, optimizing production of IVT mRNA, standard procedure of IVT mRNA purification, efficient mRNA delivery platforms, as well as optimal route of administration, which will be discussed in detail following [33].

As aforementioned, despite of the inherent self-adjuvanticity of mRNA vaccines, it is sometimes not enough to elicit comprehensive protective immunity. Therefore, an increasing number of studies have attempted to test possible adjuvants of mRNA vaccine systems to induce an adaptive immune response. Overall, adjuvant design of mRNA cancer vaccines can be categorized into three strategies [34]. The most conventional method is that adjuvants and mRNA-encoding antigens are synthesized separately and then encapsulated together into the same polymer to ensure both essential components are delivered into the same antigen-presenting cell. It is illustrated that saRNA vaccines formulated with traditional adjuvant MF59 (Novartis) and cationic nanoemulsion (CNE) to increase the delivery efficiency and enhance the immunogenicity in various models [35, 36]. The second strategy is the integration of adjuvant into the mRNA packaging material (e.g. ionizable lipids in lipid nanoparticles) for the simplified production. The principle of this method is based on the natural immune-stimulating activity of packaging material [37]. Thirdly, the most innovative and simple method currently is the adjuvants fuse with the mRNA sequence itself, so that the delivery efficiency of the packaging materials and the immune-stimulating effect of the adjuvant molecules can both be maximized [38].

Based on different strategies and mechanisms for adjuvant design, various methods have emerged. The first is agonists of TLRs, which are critical innate immune receptors, encoding different proteins (e.g. TLR1-5, TLR7, TLR8 and TLR9) to recognize different PAMPs to further activate downstream signaling pathways and initiate immune responses. This kind of adjuvants includes MPLA (a TLR4 agonist) and imiquimod (a TLR7 agonist), which have been approved by FDA for cancer treatment [39]. Similarly, Tri-palmitoyl-S-glycerylcysteine peptide (Pam3) is another well-known lipid adjuvant, which targets TLR 1 and 2. By the integration Pam3 into LNP containing ovalbumin (OVA) mRNA, it has been demonstrated that the synergistic effect of Pam3-LNP can effectively improve the cancer prevention effect of mRNA vaccines [40]. Secondly, one of the most promising immune adjuvants is agonists for the stimulator of interferon genes (STING), such as cyclic GMP-AMP (cGAMP) belonging to cyclic dinucleotides (CDNs). Mechanistically, these STING agonists can promote the activation of STING signaling in APCs and induce the IFN I secretion, thus promoting the subsequent proliferation and activation of T lymphocytes. Currently, new generation of formulations have been developed to promote tumor antigens delivery and STING signaling activation synergistically by the application of nanoparticles, such as iron oxide nanoparticles (IONPs), preferred iron nanoparticle (PEIM). For instance, IONPs can achieve a 16-fold dose-sparing effect in human STING haplotypes, while PEIM triggers an antigen-specific CD8+cytotoxic

T lymphocyte response 55 times higher than soluble antigens to induce effective and durable anti-tumor immunity [41, 42]. Thirdly, certain immunomodulatory molecules also have adjuvant activity. This can be exemplified by TriMix, a cocktail mRNA encoding three immune-modulatory molecules (active TLR-4, CD40-L and CD70). With functions of promoting DC maturation and enhancing cytotoxic T lymphocyte responses, TriMix has been incorporated into numerous vaccination studies. In stage III or IV melanoma patients, the administration of TriMix together with other tumor-antigen mRNAs showed favorable tumor response rates and achieved a durable clinical relief [43]. The fourth category of adjuvant is some mRNA delivery vehicles, such as cationic lipids and protamine. In 2013, mRNA vaccines were immunized with cationic lipids DOTAP/DOPE. The results showed that DOTAP/DOPE-loaded mRNA can induce more pro-inflammatory cytokines and type I IFN secretion, indicating DOTAP/DOPE can strengthen the adjuvant effect and the efficacy of mRNA vaccines to a certain extent. Furthermore, researches also demonstrated that protamine also has intrinsic adjuvanticity. In this context, the RNActive vaccine platform (CureVac AG) was designed using mRNA and protamine complex as the adjuvant to arouse strong immune responses [44]. Although RNActive vaccines have good tolerability and immunogenicity, as well as high levels of safety and flexibility in many preclinical and clinical trials, the suppression of protein translation was observed owing to IFN I activation after application. To overcome this limitation, an updated nanoparticle system was developed more recently, which showed potent immune responses targeting specific antigens and highly effective antitumor activities [45]. More recently, RNAdjuvant is an innovative TLR-7/8/RIG-I agonist based on non-coding ssRNA developed by CureVac. In the ssRNA, poly U repeats can be stabilized by a cationic peptide. Mechanically, the RNAdjuvant induces neutralizing antibodies by TLR7-dependent activation of markers on DCs and the production of IFN I. It has been demonstrated that RNAdjuvant can upregulate CD80, CD86 and HLA-DR in circulating DCs and promote CD4+T cell activation, resulting in a strong anti-tumor activity. The adjuvant effect of RNAdjuvant on different cancer vaccines was under further investigation [46]. Finally, it should be emphasized that adjuvant should be used with caution as it could be counterproductive due to their tight interaction with the innate immunity pathway, especially when combining mRNA design and immunostimulatory molecules. The dilemma can be resolved, at least in part, by alternative strategies, such as the use of innate immune inhibitors to bypass the type I IFN response [45].

Target selection of mRNA cancer vaccines

It is a simple idea that the induction of robust tumor-selective responses can be mobilized by mRNA-base vaccines to eliminate the existing cancer cells. Definitely, the first key step in cancer vaccines development is the selection of a proper target, which should have high tumor specificity and induce strong and controllable antitumor responses. Currently, mRNA cancer vaccines are classified as tumor antigens (TAAs and TSAs), cytokines, antibodies and immunomodulatory receptors based on the final product types (Fig. 3).

TAAs and TSAs

To date, administration of TAAs-expressing mRNAs is the most practical application of mRNA vaccines in oncology. TAAs are self-antigens that are abnormally expressed in tumor cells but can also present in normal cells at certain levels. The common candidates of TAAs include oncofetal antigens, cancer-testis antigens (CTAs), and overexpressed self-antigens. The first attempt for TAA mRNA vaccines in cancer therapy was performed using carcinoembryonic antigen (CEA) as a target in 1995 [18]. Due to the autogeneous feature, TAAs are characterized by weak tumor specificity and immunogenicity, as well as high immunologically tolerated with limited vaccine potency. To circumvent these disadvantages, the utilization of mRNA encoding multiple TAA mixtures is growing in popularity to enhance cancer vaccine efficacy for various types of cancers. The FixVac BNT111 vaccine from BioNTech is one example of an mRNA cancer vaccine that targets four melanoma TAAs. The results of clinical trials showed strong potential for the induction of antitumor immune responses. Besides, other mRNA vaccines targeting multiple TAAs are under development and testing, such as BNT112 for prostate cancer (encoding a fixed set of five prostate cancer-associated antigens), BNT113 (encoding two oncoproteins, E6 and E7), BNT114 (a mixture of TAA mRNAs encoding breast cancer antigens), BNT115 (a mixture of three ovarian cancer antigen mRNAs) and BNT116 (encoding a fixed set of antigens frequently expressed in NSCLC). Likewise, a series of RNActive vaccines encoding TAAs (e.g. CV9103, CV9201 and CV9202) for cancer treatment have been developed by CureVac AG, another mRNA drug company [47]. All these preclinical and clinical trials have been summarized in the following sections.

In contrast, TSAs (also known as neoantigens), which mainly derived from extensive mutations of caner genome, are exclusively expressed in tumor cells. Because they are recognized as foreign materials, TSAs exhibit high specificity and improved immunogenicity, as well as weak central and peripheral immune tolerance [48]. Logically, TSA vaccines are more attractive and ideal targets for cancer vaccines. Due to these prominent advantages,

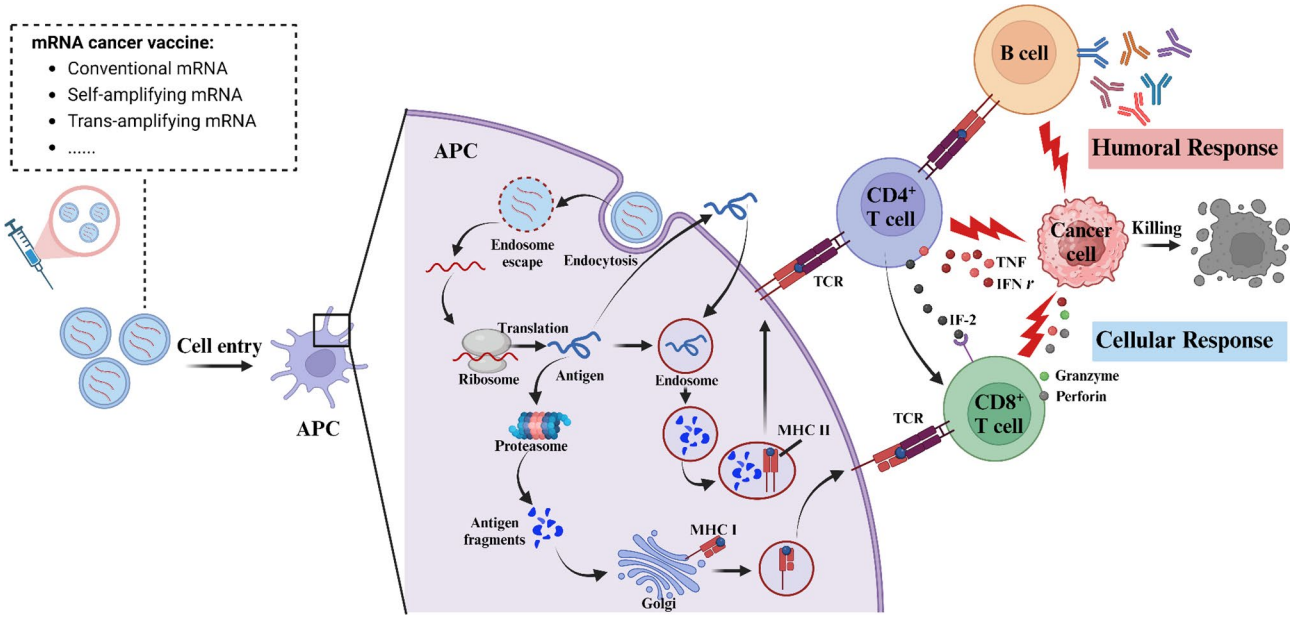

Fig. 3 The mechanism of mRNA cancer vaccines. The process of antigen presentation and the activation of cellular and humoral immunity after mRNA vaccine injection are illustrated. Briefly, the mRNA vaccine can be captured by APCs where mRNA is translated into protein and processed by proteasome in the APCs. Then it is presented by MHC I or MHC II molecules to CD8 + T cells or CD4 + T cells, respectively. Additionally, CD4 + T cells can further coactivate specific B cells. Eventually, cancer cells can be eradicated by the activation of both cellular and humoral responses. Figure created with BioRender (biorender.com). Abbreviations: APC, antigen-presenting cell; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; TCR, T cell receptor; TNF, tumor necrosis factor

TSA-based vaccines are undergoing rapid progress and several clinical neoantigen vaccines are under evaluation at present, including BNT122 from BioNTech (phase II) and mRNA-4157 from Moderna and Merck (phase III). More inspiringly, the UK government reached an agreement with BioNTech last year to treat up to 10 000 cancer patients with personalized mRNA vaccines by 2030 [49]. In addition, accumulating evidence confirmed that whether a TSA can be used as a therapeutic target depends on several critical factors, such as enough expression level, potent antigenicity without central tolerance, efficient process and presentation by APCs, as well as high binding affinity to molecules of major histocompatibility complex (MHC). Some studies also confirmed a correlation between TSAs and antitumor immune responses. For instance, data from The Cancer Genome Atlas (TCGA) about 18 solid tumors showed a positive correlation between the expression of genes related to the cytotoxic activity of T cells and the number of neoantigens in each tumor. Analysis of RNA-seq data from TCGA showed that a high level of immunogenic mutant epitopes was associated with improved patient survival [50]. Interestingly, Scumacher et al. reported that tumors with a greater mutational load (>10 somatic cell mutations/10^6 bases) are more likely to form immunogenic neoantigens, and tumors with a less mutational (<1 somatic cell mutations/10^6 bases) are less likely to form immunogenic neoantigens. They also found that the mutational load for most cancers is ranging from 1 to 10 and neoantigens can be formed and further recognized by T cells generally [51]. In this context, it should be reminded that for cancers with a lower prevalence of somatic mutations and frequency of mutant neoantigens, such as prostate cancer, the identification and application of TSAs may be difficult and even unpractical [52]. Another issue should be concerned is further optimization needs to be conducted to reduce the cost and complexity of TSA vaccine, due to uniqueness of neoantigens to each patient and cancer type [53].

mRNA-encoded immunomodulators

Generally speaking, there are two approaches in mRNA-based cancer vaccination, which are interrelated and interact with each other. In addition to the therapeutic immunization with mRNA encoding antigens (TAAs or TSAs) in cancer patients as aforementioned, another meaningful approach is based on the strategy that mRNA can be used as a vehicle to delivery immunomodulators, such as cytokines and stimulatory molecules. Although these immunostimulants sometimes are not regarded as cancer vaccines, their coadministeration with cancer vaccines or other immunotherapeutic agents can really augment humoral and cellular response. Therefore, anyway, preclinical and clinical researches about mRNA-encoded immunomodulators have become an important direction for mRNA cancer vaccine.

Cytokines are secreted signaling proteins playing essential roles in the initiation, maintenance and regulation of immune responses. In the context of cancer, the unique ability of cytokines has garnered clinical interest for a long time. Thanks to a growing insight into the complex network of immune cells and stromal components in the tumor microenvironment (TME), it has been widely accepted that the overall effect of the cytokine milieu is determined by the relative ratio of various cytokines with anti-tumorigenic and pro-tumorigenic properties [54]. Given the flexible profile of mRNA vaccine, multiple cytokines and other immune-related factors can be introduced simultaneously into encoded sequences of mRNA, thereby enhance immune system responses and reshape the TME through diverse pathways. To date, commonly used antitumor cytokines include interferons, interleukins (ILs), and tumor necrosis factors (TNFs) with various functions [55]. Over the last decade, several clinical trials of mRNAs encoding cytokines have been conducted by Moderna and BioNTech. Among them, mRNA-2416 (mRNA-encoded OX40L), developed by Moderna in 2017, was the first clinical trial expressing mRNA-encoded immunostimulant. Subsequently, an escalatory vaccine named as mRNA-2752 encoding OX40L, IL-23, and IL-36γ pro-inflammatory cytokines was further investigated in solid tumor patients [56]. Meanwhile, BioNTech developed the BNT151-153 candidates for clinical trials. These cytokine-based products demonstrated promising clinical results, such as amplified T cell responses and improvement of tumor-mediated immunosuppressive effects [57]. The related clinical trials have been summarized in the following sections.

Other targets encoded by mRNA in cancer immunotherapy

It is well-known that the efficacy of cancer vaccines or immunotherapy can be affected by through various mechanisms, such as tumor evasion and resistance. In this context, a plethora of attempts have been made through using other candidates encoded by mRNA to achieve synergistic therapeutic effects with mRNA vaccines. These additional targets comprise various antigen receptors, diverse antibodies, as well as vital tumor suppressor genes (TSGs). For tumor immunotherapy, presenting TAAs/TSAs to T cell receptors (TCRs) by specific intermediary (e.g. MHC-I) is an initiator of CD8+-cytotoxic-T-lymphocyte (CTL) activation. As a result, it is plausible that upregulation of MHC-I on tumor cell surfaces and/or application of genetic-engineering T cells with cancer-specific TCRs or chimeric antigen receptors (CARs) can prevent tumor escape from the immunologic surveillance and improve the tumor's immunogenicity [58, 59]. For instance, a revolutionary T-cell therapeutic modality under exploration is a T-cell reprogramming strategy through the delivery of antigen receptors via mRNA to T cells in vivo. Also, the co-transfection with immunostimulatory ligands and receptors introduced by mRNA can enhance dendritic cells (DCs) maturation and T-cell priming capacity. Next, monoclonal antibodies (mAbs) are currently accepted as a primary therapeutic method in the field of cancer immunotherapy. Many studies have shown that fully bioactive mAbs can be produced in vivo by delivering mRNA. Rituximab, a clinically approved IgG1 that targets CD20, is a classical example of an mRNA-encoded mAb. With rapid progress in this field, mRNA-based expression of bispecific antibodies (bsAbs) that forms a bridge between tumor cells and T cells provides another promising approach to induce target-dependent T cell activation and enhance anti-tumor T cell activity [60]. In addition, several preclinical studies have also shown the feasibility of using mRNAs-encoded TSGs (e.g., PTEN and p53) to treat tumors. Lin and colleagues showed that the PTEN-encoding mRNA can upregulate CD8+T cells and proinflammatory cytokines in the immunosuppressive TME and downregulate Tregs and MDSCs, and produced potent antitumor effects against melanoma and prostate tumors combination with an anti-PD-1 antibody [61]. Similarly, the p53 tumor suppressor pathway has been reported to modulate the interactions between tumor cells and immune cells via regulation of cytokines and chemokines and mRNA cancer vaccines encoding p53 and tumor antigens (e.g., survivin, hTERT, neoantigens) are currently in clinical trials [62].

Optimization of mRNA translation and stability

After selecting the target antigen, another critical factor is the optimization of the mRNA sequence in mRNA vaccine design, which can make the mRNA more stable, highly translatable, unwanted immunogenicity as well as high delivery efficiency.

5′ cap and modification

In eukaryotic cells, 5' cap is a characteristic structure in functional mRNAs formed by linking m^7G to the 5'-end of transcripts via a 5'-5' triphosphate linkage. It is vital for many mRNA functions, such as covering protection against exonucleases, enhancement of mRNA translation efficiency, and nuclear export of endogenous precursor mRNA. The canonical 5' cap structure is an inverted 7-methylguanosine (m^7G) in endogenous mRNA commonly referred to as 'Cap 0' ($m^7GpppNp$). At the basis of cap 0, cap 1 ($m^7GpppNmpNp$) and cap 2 ($m^7GpppNmpNmp$) can be further formed by the methylation of 2'-OH on the first and the second nucleotide, respectively [63]. For IVT mRNA, the triphosphate moiety at its 5' end can be recognized by cytoplasmic PRRs, such as RIG-1 and IFIT, triggering IFN I-mediated innate immune response to inhibit translation and protein

synthesis [64]. In this context, the remove of the triphosphate and the addition of 5' cap should be conducted to prevent the RNA from being identified as non-self nucleotides.

At present, there are two common methods to cap the IVT mRNA strand, the transcriptional (chemical) and posttranscriptional (enzymatic) capping [65]. Transcriptional capping is the addition of a cap analogue ((m^7GpppG)) to the reaction for co-transcription via bacteriophage polymerases. Yet, this method has a risk that the cap analogue may bind to the mRNA in the reverse direction, resulting in a decrease in the translation efficiency. To circumvent the problems aforementioned, an innovative technique known as anti-reverse cap analog (ARCA) was developed to allow the polymerase to add to the nucleotide strand in the correct orientation. Unfortunately, the capping efficiency of ARCA remains low (60–80%) and at least 20% of mRNAs fail to be capped after transcription [66]. In 2018, CleanCap™, a next generation co-transcriptional capping with capping efficiency to nearly 90–99%., was developed by TriLink BioTechnologies to overcome the issues associated with ARCA [67]. For post-translational capping, the most widely method is the vaccinia capping system through vaccinia virus capping enzyme (VCE) to produce IVT mRNA with cap 0 [68]. Relatively speaking, VCE capping offers specificity but at higher cost and complexity [69]. To date, IVT mRNA with cap 1 can be obtained through further processing by VP39 in vaccinia viruses or VP4 in Bluetongue viruses directly [70, 71]. Nevertheless, it must be noted that neither transcriptional methods nor posttranscriptional capping can guarantee all the RNA strands can be capped entirely. In addition, because the cap1 structure is now most commonly used for capping mRNA vaccines, it is necessary for the capping product to make an accurate quantification and classification. To this end, CapQuant is an important detection tool based on a system-level mass spectrometry technology that can accurately quantify various types of 5'-cap [72]. Collectively, given the essential roles of 5' cap, its suitable modification is a critical factor in optimizing the design of mRNA vaccines.

3′ poly (A) tail and modification

The poly (A) tail, found in most eukaryotic mRNAs, is a critical posttranscriptional modification of majority mRNA that significantly contributes to its stability, export, and translation. In vitro, Poly (A) tailing of mRNA can be accomplished either by enzymatic polyadenylation through recombinant Poly (A) polymerase or by transcription according to tail in a designed DNA template with cloning Poly (T). The major difference between the two methods is that the former Poly (A) tail length is varying, while the latter allows for a defined Poly (A) tail length as needed and is therefore preferred [73]. A suitable length of Poly (A) is crucial, as many studies demonstrated that the length of the Poly (A) tail can largely affect the stability and translational activity of mRNA. Generally, mRNA with longer Poly (A) tail exhibits a higher stability and translation efficiency. However, further studies implied that different cells may have different preferences. For instance, the optimal length of Poly (A) in human primary T cells are 300 nucleotides, while in human monocyte-derived DCs are only 120–150 nucleotides [74, 75]. Surprisingly, Lima and coworkers reported a paradoxical finding that short Poly (A) tails are a conserved feature of highly expressed genes using Poly (A) tail sequencing, indicating some unknown mechanisms may be involved [76]. With regard to IVT mRNA, Poly (A) tails with different lengths, ranging from 60 to 150 nt, have been tested in various cell lines. The observations demonstrated that the optimal tail length of IVT mRNAs requires adaptation to a specific case, including the intrinsic properties of IVT mRNAs and the cytoplasmic environment [77]. Additionally, many studies have shown that a number of modification sites exist in the Poly (A) tail region, such as uridylation and guanylation of the Poly (A) tail. The former modification was found to promote mRNA decay, while the latter modification could protect mRNA from rapid deadenylation. Therefore, modification of the Poly (A) tail should also be an important consideration in the design of mRNA vaccine. To date, there have been several methods developed for deciphering sequence features of the Poly (A) tail, such as Poly (A) inclusive RNA isoform sequencing (PAIso-seq), tail sequencing (TAIL-seq), and Poly (A)-tail length profiling by sequencing (PAL-seq) [78, 79]. To sum up, further work is needed to elucidate the underlying mechanisms of Poly (A) tail in the functional regulation of mRNA and mRNA vaccines can be better designed for efficient protein expression.

Modification of 5′- and 3′-UTRs

UTRs, flanking the open reading frame (ORF), possess various regulatory elements that affect many functions of mRNA, including stability, translation and subcellular localization. Due to the in-depth insight into the regulatory mechanisms in UTRs, it has been confirmed that the sequence, length and secondary structure of UTRs all affect mRNA functions. As such, overall considerations should be taken during the optimization of vaccine design. Currently, designed 5' UTR sequences are mainly derived from genes such as α/β-globin and heat shock protein 70 (Hsp 70). Several rational standards have been recommended for the design of 5' UTR: ① To avoid disturbing the translation initiation, canonical start codon (AUG) and suboptimal start codon (CUG) cannot exist in 5' UTR. ② Highly stable secondary structures should be avoided as these structures can prevent ribosome

recruitment and codon recognition ③ A short and loose 5' UTR may be more ideal and conducive for mRNA translation [80, 81]. Similarly, the 3' UTRs also have regulatory sequences and play a pivotal role in regulation of mRNA functions. The sequences of globin gene from Xenopus laevis or humans are commonly used in many preclinical and clinical studies involving IVT mRNA. Because adenylate-uridylate-rich elements are the most common mRNA stability determinants in mammals, the adjustment of AU-enriched sequences and GU-enriched sequences can avoid the form of unstable structures and prevent mRNA from degradation. The other essential strategies about 3' UTRs design should also be considered, such as miRNA binding sites and proper length. Moreover, it should be highlighted that UTRs impact different cell types variably, requiring tailored optimization for specific target cells. Finally, recent evidence showed various forms of RNA modifications can be found both in 5'-UTR sequences and 3'-UTR sequences, which make the design of UTRs more complicated. For example, while N1-methyladenine (m^1A), N6-methyladenine (m^6A), N6, 2'-O-dimethyladenosine (m^6Am), 5-methylcytosine (m^5C) and pseudouridine (Ψ) were found in 5'-UTRs, m^6A, m^5C and Ψ are prevalent within the 3'-UTRs. Inspiringly, the rapid advancements in machine-learning-based framework and high-throughput-screening technology have facilitated the design work because of its iterative optimization capability and open-source linkage of data and algorithms [82]. Altogether, UTRs performance may be dependent on many factors, and customized design of UTR for specific target is necessary.

Optimization of CDS

The coding sequence (CDS) is the core of the mRNA, because the ORF in CDS not only can determine the identity and structure of the protein synthesized, but also has remarkable affect on the translation efficiency. Similarly, the importance of ORF design in mRNA vaccines is paramount owing to its direct influences on the production of the target antigen. During the optimization of CDS, the first issue should be concerned is about the codon preference, which means the optimal codons can be used more frequently than the rare codons [83]. Therefore, one common strategy is to use optimal codons (e.g. frequently used synonymous codons with abundant cognate tRNA in the cytosol) as replacements for rare codons to promote mRNA translation and increase the protein yield. However, it is worth mentioning that high translation rate is not always beneficial, because a low translation rate is required for some proteins to make correct and effective folding. In contrast, the accuracy of translation is more important than simply focusing on the translation speed [84]. Another form of sequence optimization is the enrichment of the GC content. Studies showed that the translated rates of GC enriched sequences can be 100-fold higher than those of low GC sequences, while uridine content is negatively correlation to protein expression. The facilitation to protein expression may attribute to the augment of the mRNA stability and enhancement of steady-state mRNA levels [85]. Furthermore, it has been reported that certain position of codon pairs (e.g. G/C or A/U) is associated with mRNA stabilization and translation, known as codon pair bias. For example, GC3 (G or C at the third position of codons)-rich mRNAs have a higher ribosome reading rate and protein expression efficiency than AU3-rich mRNAs, indicating GC3 and AU3 content can be used to some extent as markers to reflect the properties of mRNAs [86]. Besides, unanticipated secondary structures may be generated occasionally by inappropriate codon optimization, which can affect the authenticity of ribosome scanning, resulting in erroneous wobble pairing and decreased protein expression. Also, it should be emphasized that highly stable secondary structures and hairpin loops near the start codon should be avoided, since more energy is required to unfold during translation initiation and then slows down the translation rate [87]. Accordingly, the optimization of both mRNA and secondary structures may have a synergistic effect in terms of accelerating protein expression. Finally, CDS in mRNA can be optimized by incorporating specific RNA modifications to improve some properties of mRNA, such as stability and translation accuracy. Generally, preferred nucleotide modifications include m^5C, Ψ and N1-methyl pseudouridine ($m^1\Psi$) [78]. In conclusion, the optimization of CDS should be considered comprehensively and carefully monitored because of multiple factors involved to achieve rational regulation of translation efficiency.

Purification

As aforementioned, multiple contaminants can be generated through IVT process, leading to abolish of mRNA translation, innate immunity activation and strong immunogenicity of IVT mRNA. Among these abnormal products, short RNAs may originate from abortive initiation, while dsRNA can be generated by self-complementary 3' extension. In addition, it has been demonstrated that, under standard conditions, entire mRNA translation depends more on the purity and sequence composition, rather than nucleotide modifications and the length of the mRNA. For instance, Karikó et al. reported that the removal of these RNA contaminants result in remarkable decrease of IFNs induction and inflammatory cytokines release, ultimately leading to 10- to 1000-fold increase in protein production in human primary DCs [88]. In this context, the removal of these byproducts is indispensable

for IVT mRNA vaccines. Originally, attempts have been carried out to minimize the amount of dsRNA by the adjustment of reaction conditions, such as low Mg^{2+} concentration or elevated temperatures during IVT. Currently, the scalable purification of IVT impurities was commonly performed by high-pressure liquid chromatography (HPLC). However, HPLC removal of mRNA is usually high cost and low yield (<50%). More recently, Baiersdörfer and coworkers reported a rapid and cost-effective purification method, which utilized the selective binding of dsRNA to a cellulose powder in ethanol containing buffer to remove up to 90% of dsRNA [89]. In addition to IVT, mRNA can also be synthesized on-scale by solid phase method to completely get rid of dsRNA contaminants. For details, the literature of Shivalingam and colleagues was referred to [90].

Delivery platforms of mRNA vaccines

As an exogenous mRNA artificially synthesized in vitro, mRNA vaccines must enter the cytosol of target cells or tissues to functionalize. Nevertheless, this process is hindered by some inherent characteristics of mRNA itself and internal milieu, which can make great challenges to the application of mRNA vaccines. Correspondingly, as a key aspect of mRNA vaccines, various delivery systems were explored to overcome these limitations and enhance the efficacy of mRNA vaccines. As viral vehicles have been reviewed extensively elsewhere, an overview about some common non-viral, and cell-based platforms for mRNA vaccine delivery is presented in this section (Fig. 4).

Dendritic cells-based delivery systems

DCs are one of the most potent APCs of immune system. DCs not only can stimulate T cell-based immune through the presentation of proteolytically digested antigens to MHCs on helper T cells (CD8+and CD4+T cells), but also have the ability to transmit intact antigen to B cells to trigger humoral immunity. Moreover, it has been reported that naked mRNA can be internalized into DCs via different endocytic pathways and higher transfection efficiency can be achieved ex vivoby electroporation even without a vehicle. Owing to these unique abilities, DCs are an ideal candidate frequently used delivery vehicle for mRNA vaccination. As early as in the 1990s, ability of DCs to reliably primed T cells in situ has been identified [91]. In 1996, the pioneering study demonstrated that DCs pulsed with mRNA elicited potent immune responses against the tumor antigen [92]. Generally, DCs can be loaded with mRNA both ex vivoand in situ. For the ex vivocondition, autologous DCs from peripheral blood are first processed after the maturation and antigen-encoding mRNAs loading in vitro, and then the engineered DCs are re-infused back into the recipient to start the antigen-specific immune response. For the in situ context, DC transfection can be realized by directly injecting antigen-encoding mRNAs complexed with TriMix into lymph nodes. The first clinical trial of TriMix-DCs vaccine was performed in patients with advanced melanoma [59]. However, the production of DCs-based vaccines cannot meet the huge quantity demand of mRNA vaccine for some treatments owing to the its limited number in natural circulation and labor-consuming production process, such as the isolation of leukocytes from blood, in vitro culture, promoting cell differentiation and antigens loading [93].

Naked mRNA-based delivery systems

The injection of naked mRNA molecules into cells or tissues, with advantages of easy production and cost-effectiveness, was one of the earliest approaches for mRNA vaccines. From the pioneering attempt from Wolff et al., numerous studies using naked mRNA provided some encouraging results in animal models and (pre)clinical trials over the past three decades [15]. Despite it is the case that certain cells can take up naked mRNA, the uptake efficiency of naked mRNA is too low (<1%) to have a significant effect on host cells. It has been found that the administration route can affect delivery efficiency of naked mRNA in vivo obviously. For example, some researchers have suggested that immature DCs in the lymph nodes or dermis can selectively take up naked mRNA via micropinocytosis [94]. Based on this, the injection of naked mRNA vaccines containing encoded antigens is mainly via intradermal or intranodal manners. Since naked mRNA is formulated only in buffer and without the protection of carries, naked mRNA vaccines remain limited by the short extracellular half-life due to rapid degradation by ubiquitous RNAases. Moreover, it can only induce transient protein expression, limiting the durability for treatment effectiveness [95].

Lipid-based mRNA delivery systems

The features of high biocompatibility and selective charge make lipid materials become one of the most appealing platform for mRNA vaccine delivery. Overall, lipid-based mRNA delivery systems can be divided into two major classifications, liposomes and lipid nanoparticles (LNP). To some extent, liposomes can be considered as an early version of LNPs. Liposomes are small vesicles at the nanoscale mimicking the cell's membrane. They are made up of one or more lipid bilayers with an internal cavity that can hold hydrophilic substances, such as mRNAs. In contrast, LNPs are characterized by a micelle-like structure assuming homogeneous morphologies of solid spheres only with a low or minimally aqueous internal core. The discontinuous lipid bilayer on LNPs qualify them as lipid vesicles and the nucleic acids can be

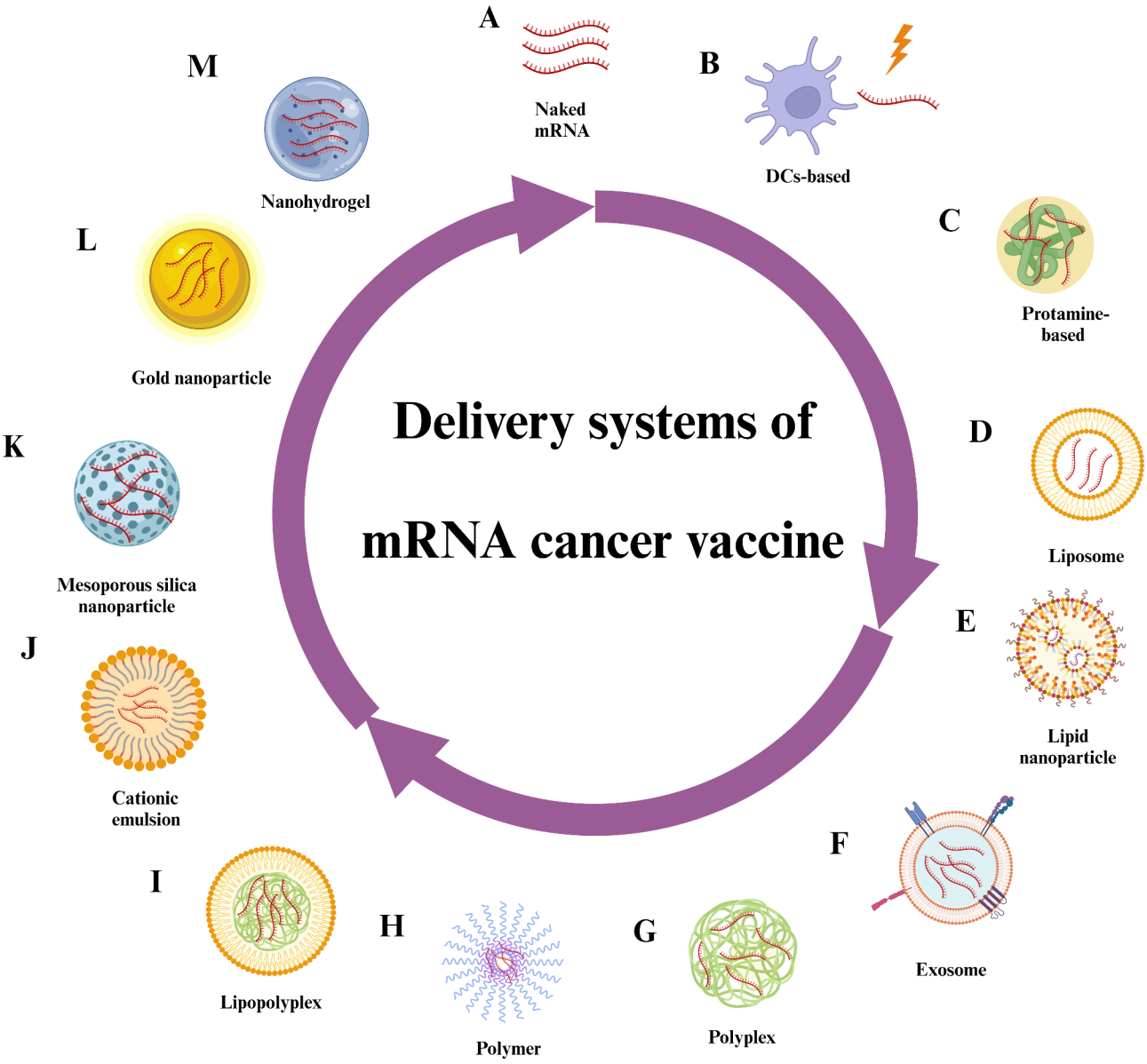

Fig. 4 Various delivery systems of mRNA cancer vaccines. Commonly used delivery methods and carrier molecules for mRNA vaccines are shown. (**A**) Naked mRNA. (**B**) DCs-based. (**C**) Protomine-based. (**D**) Liposome. (**E**) Lipid nanoparticle. (**F**) Exosome. (**G**) Polyplex. (**H**) Polymer. (**I**) Lipopolyplex. (**J**) Cationic emulsion. (**K**) Mesoporous silica nanoparticle. (**L**) Gold nanoparticle. (**M**) Nanohydrogel. Figure created with BioRender (biorender.com)

entrapped within the internal core, rendering them suitable for stable and efficient encapsulation of genetic payloads [96]. With advancements in the lipid-based delivery techniques for mRNA vaccines, many innovative derivatives were developed.

Initially, liposomes composed of cationic lipid are the first generation of carriers used for mRNA delivery in (pre)clinical trials. DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium-propane) and DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) are first used as RNA carrier in 1989 and become the most common cationic lipids for mRNA delivery to date. These cationic lipids can not only bind anionic mRNA via electrostatic interaction but also fuse the membrane to promote cellular uptake and endosomal escape. To form and maintain the lipid bilayer structure, lipsomes also have some helper lipids, such as 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphatidyl-ethanolamine (DOPE), and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). Despite the high efficiency of mRNA delivery, further studies revealed that the permanent charge DOTMA/DOTAP carried resulted in high cytotoxicity and limited their potential applications [97]. As the second-generation cationic lipids, ionizable lipids were introduced to overcome the shortcomings of DOTMA/DOTAP due

to their unique pH-dependent properties [98]. On one hand, ionizable lipids can present as electrically neutral at physiological pH, which is necessary for reducing toxicity and improving safety. On the other hand, they can become positively charged at the acidic environment, which facilitate the processes of mRNA encapsulation, uptake, and endosomal escape. The first ionizable lipids utilized for RNA transport were 1,2-Dioleoyl-3-dimethylammonium-propane (DODAP) and 1,2-Dioleyloxy-3-dimethylaminopropane (DODMA) [99]. Currently, many novel ionizable lipids can derived from synthesis based on rational design and high throughput screenings of combinatorial libraries [82]. While mRNAs bind to liposomes, they would be embedded between the lipid bilayer and then self-assemble into lipoplex (LPX), a form of liposome derivative. Several cancer vaccines based on LPX were performed and evaluated (See the following sections).

For lipid-based vehicles, LNPs are the most popular and the first option for the mRNA delivery so far, especially after the great achievement associated with the COVID-19 vaccine. The success and potency of LNPs for mRNA delivery rely on several essential components, including cationic/ionizable lipids (40–50%), auxiliary phospholipids (10–12%), polyethylene glycol (PEG) (1–2%), and cholesterol (38–45%) [100]. Just these key components provided LNPs better kinetic stability and a more rigid morphology than liposomes. Among these, ionizable lipids play a key role for LNPs delivery platforms in protecting mRNAs from RNase degradation, fusing with the endosomal membrane, as well as releasing the mRNA into the cytoplasm. Of note, studies have uncovered that the properties of ionizable lipids can be affected by several parameters, such as tail length, unsaturation and branching. In this context, many companies have developed hundred of ionizable compounds for various applications. For example, ALC-0315 (BioNTech) and SM-102 (Moderna) were used for the COVID-19 vaccine, respectively [47]. Besides, auxiliary phospholipids and cholesterol promote the formation of lipid bilayers to encapsulate mRNA and stabilize the structure of LNP-mRNA encapsulations further, while the incorporation of PEG can decrease macrophage-mediated clearance and prevent particle aggregation to extend the half-life of LNP formulations.

With the improvements on mRNA delivery with LNPs, more and more mRNA-LNP cancer vaccines are currently in clinical trials. Despite of many outstanding advantages, there are still some disadvantages in practical applications for LNPs-based platforms. The main challenges to be overcome are most LNPs may accumulate in the liver post-administration, potentially leading to hepatotoxicity and limiting the administered dose [101]. For this reason, targeting strategies of LNP-mRNA cancer vaccines are focused on to ensure mRNA can be delivered to specific organs or cells. The best-known example is selective organ targeting (SORT), which is characterized by the addition of a fifth SORT lipid to a conventional LNP to alter the internal charge and interact with serum proteins that bind to receptors on target organ cells, and ultimately promote tissue-specific targeting [102, 103]. Although the underlying mechanisms of these SORT lipids are not fully clear, it can be anticipated that the optimization of targeted LNPs for mRNA delivery must enhance LNP performance and application in the future. Another advantage of LNPs is their self-adjuvant activity, which can enhance the immune response and improve vaccine efficiency. For instance, many studies have demonstrated that both cationic lipids (e.g. DOTAP/DOPE) and ionizable lipids (e.g. SM-102 and ALC-0315) used as LNP packaging components have natural immune-stimulating activity. Therefore, on the one hand, it is undoubted that the self-adjuvant effect of LNP is of a great advantage for mRNA vaccine and makes it more practical in application. On the other hand, however, it should also be realized that the extensive screening and optimization to find suitable packaging materials will be necessary but challenging, because the clear mechanisms of the relationship between the lipid components and the process by which LNPs exert their adjuvanticity remain unknown [104].

Protamine/Peptide-based delivery system

Due to the electrostatic interaction, the negatively charged mRNA is easily delivered by the cationic peptide. In comparison with lipid-based delivery systems, peptide-based platforms exhibit higher transfection efficiency and reduce harmful effects due to a series of advantages, such as better biocompatibility, simple synthesis, small size, rare off-target side effects [105]. The first example of peptide-based carrier is protamine, an alkali polycationic protein with resin-like structure. Spontaneously, protamine can condense mRNA through electrostatic interaction to form the protamine-mRNA complexes, and thus has been used in the delivery of mRNA vaccines for a long time. Of note, studies have revealed that protamine-mRNA complexes have the potential not only as mRNAs carriers protecting mRNAs from degradation, but also function as adjuvant to activate immune reactions [106]. However, protamine-complexed mRNA alone can only display limited protein expression and antitumor efficacy in preclinical studies. This may be due to the excessively tight conjugation between protamine and mRNA, resulting in limited dissociation and hinder efficient mRNA release. To solve this concern, RNActive was developed by CureVac AG. RNActive was designed as a two-compartment formulation with the combination of protamine-mRNA complexes (50%) and nucleoside

modified mRNA (50%) to enhance protein expression and immunogenicity [107]. The protamine complexes act only as adjuvant, while the nucleoside modified mRNA acts as antigen producer. RNActive vaccines have been evaluated in several clinical trials and some of them have shown moderate antitumor efficacy.

Cell-penetrating peptides (CPPs) are the other class of potential mRNA drug delivery systems. Most of CPPs are cationic peptides with 8–30 amino acids in length, which can spontaneously complex with anionic mRNA and interact with the negatively charged cell membranes through electrostatic interactions [108]. Although their cell-uptake mechanisms are not fully understood, it is hypothesized that the structure of CPPs with repeated arginine-alanine-leucine-alanine (RALA) motifs can be regulated by the pH of endosome, thereby, facilitating the creation of pores between membranes and endosomes to ensure the translocation of peptide and enabling the delivery of mRNA [109]. Of note, studies also found that the RALA motif in CPPs is vital for the mRNA transport into DCs and the activation of T cell-mediated immunity. Furthermore, more potent vaccination effects can be induced by some CPP-mRNA complexes compared to standard liposomal mRNA formulations [110]. Interestingly, it has been revealed that cationic peptides have higher penetration efficiency than anionic molecules since anionic residues make up the surfaces of most cancer cell [111]. The exploitation of CPPs in the delivery of mRNA is still ongoing, further research is expected.

Polyplex/Polymer-based delivery system

Polyplexes and polymers are other promising alternatives for efficient mRNA delivery. The formation of polyplexes is based on the electrostatic interactions between negatively charged mRNA molecules and positively charged polymers, such as polyethyleneimine (PEI). In contrast, some polymers such as poly-L-lysine (PLL), polyaminoesters (PBAEs) and polyamidoamine (PAMAM) dendrimer can act as a component to form polymeric nanoparticles, which can encapsulate mRNA through multiple mechanisms, involving electrostatic interactions, hydrophobic interactions, as well as covalent bonding. Among them, PEI has been most widely used for mRNA vaccine delivery [112]. Compared with lipid-based delivery platforms, the primary disadvantages of these materials are the polydispersity and biodegradability, which can lead to lower clearance rate and durable cytotoxicity [113]. To improve the tolerability and stability of the polymeric platforms, structural modifications have been evaluated, including the incorporation of lipid tails, hyperbranched groups and biodegradable moieties. For example, Haabeth and coworkers reported that mRNA can be delivered effectively by a novel lipid-containing polymer called charge-altering releasable transporters (CARTs). Furthermore, it has also been found that the mixed-lipid CARTs are more effective in transfecting lymphocytes, CD4+T cells and CD8+T cells than single-lipid CART [114]. It should been mentioned that, although cationic polymers are dominant for mRNA delivery, some anionic ones are also under exploration, such as poly lactic-co-glycolic acid (PLGA). It has been demonstrated that the addition of cationic lipid in a PLGA complex is benefit to establishing an efficient RNA-encapsulating system [115].

Exosome-based delivery system

Exosomes originate from the endosomal system within diverse cells, with diameter ranging from 30 to 150 nm. Given their natural origin, the exosome lipid bilayer shows a great biocompatibility, deep tissue penetration and low immunogenicity when compared to other synthetic delivery systems [116]. Due to these unique characteristics, exosomes have been emerging as a promising delivery system for RNA therapeutics, including mRNA vaccines, which can be exampled by the success of exosome-mediated mRNA delivery for COVID-19 vaccination [117]. Currently, various genetic materials have been loaded into exosomes, including adenoviruses, long noncoding RNAs and circular RNAs. Meanwhile, many efforts are ongoing to load exosomes with sufficient amounts of mRNA for clinical use [118]. Furthermore, some recent studies have claimed that mRNA-loaded exosomes are much more efficient than mRNA-loaded LNPs in delivering mRNA to target cells in vitro. However, some challenges hinder the applications of exosomes as optimal delivery platform, such as identification of parent cells, loading efficiency, structural stability, as well as sufficient yield.

Other delivery systems

In addition to delivery platforms discussed above, other mRNA vehicles have been explored. Inorganic nanoparticles have been extensively researched for mRNA delivery, mainly including mesoporous silica nanoparticles and metal nanoparticles (e.g. iron oxide, calcium phosphate and gold nanoparticles). For these inorganic nanoparticles, the most advantages in drug delivery involve their tunable geometry and size [119]. A representative example of inorganic nanoparticles is the potential application of gold nanoparticles in mRNA delivery [120]. Another promising vehicle for mRNA delivery is nano-hydrogel. Studies showed that nano-hydrogels have better biocompatibility and higher mRNA expression efficiency compared to commercial liposomes. Due to their good biocompatibility and stability, nano-hydrogels represent a promising alternative for mRNAs delivery in vivo [121]. More recently, CNEs were explored as an mRNA delivery system, in which cationic lipid in the formulation plays a crucial role in the complexation of mRNA

through electrostatic interactions [122]. Researchers have shown that the CNE-mRNA vaccines were well tolerated and immunogenic in multiple preclinical models. More importantly, it is demonstrated that CNEs have the ability to prevent the aggregation of particles in biological fluids, which is the major limitation of liposomes, indicating significant potential in human clinical evaluation. The details about CNEs have been reviewed comprehensively, such as composition, preparation methods, and biological properties [123].

Collectively, the exploration and optimization of mRNA-delivery systems are of great importance for mRNA vaccines development. It should been note that each mRNA delivery system has its own features and limitations, and the choice of delivery system depends on various factors, including the vaccine design, specific tumor type and desired immune response. The advantages and disadvantages of different delivery platforms were summarized in Table 1. Further studies are needed to improve their efficiency, safety, and clinical applicability.

Administration route of mRNA cancer vaccines

Taking into account the diverse anatomical and physiological characteristics of vaccination sites, administration route is another crucial factor for the distribution and efficacy of mRNA vaccines in vivo. Generally speaking, the administration of mRNA vaccines can be classified into two manners overall: ex vivoloading of mRNA into DCs and direct injection of mRNA with or without a carrier [124]. In detail, the mRNA cancer vaccines can be delivered through diverse administration routes, mainly include intravenous (IV), intramuscular (IM), subcutaneous (SC), intradermal (ID), intranodal (IN), and intratumoral (IT) injection [4, 125]. The common administration routes of mRNA cancer vaccines were shown in Fig. 5.

Until now, the most frequently used routes for mRNA vaccination include IV, IM and SC. As a systemic delivery, IV injection allows for a larger volume for administration and has been performed in many clinical trials. All in all, intravenous administration has been shown to be safe, well-tolerated and permissive for repeated dosing to maintain immunity against tumor cells. On the other hand, several risks and hurdles have to be considered for mRNA vaccines after IV implement, such as the systemic toxicity and possible distribution in vivo. For example, it has been reported that predominant homing to the liver was observed owing to the unique features of liver vessels and receptor-mediated uptake by hepatocytes. Consequently, immune-mediated hepatitis or hepatic toxicity can occur because of undesired protein expression [126].

Table 1 The comparison of various delivery systems for mRNA cancer vaccines

Delivery platform	Description	Advantages	Disadvantages
DC-based	Dendritic cells with loading mRNA for antigen expression and presentation	-High expression -Efficient transfection -Targeted delivery	-Complex production process -Costly -Instability
Naked mRNA-based	mRNA introduced with direct injection or electroporation	-Easy production -Cost-effectiveness	-Low efficiency -Limited immunogenicity -Prone to degradation
LNP-based	mRNA loaded with LNP for delivery	-High transfection efficiency -Biocompatibility	-Potential hepatotoxicity -Content leakage
Protamine/Peptide-based	mRNA conjugated with protamine or ell-penetrating peptide for delivery	-High transfection efficiency -Biocompatibility -Simple synthesis -Low off-target side effect	-Limited protein expression -Moderate antitumor efficacy -Low specificity
Polyplex/Polymer-Based	mRNA complexed or encapsulated with polymers for delivery	-High transfection efficiency -Physical stability -Modifiability	-Low clearance rate -Durable cytotoxicity
Exosome-Based	mRNA loaded with exosomes for delivery	-Biocompatibility -Targeted delivery	-Loading efficiency -Structural stability -Insufficient yield
Inorganic nanoparticles-based	mRNA loaded with inorganic nanoparticles for delivery	-Modifiability -Stability -High loading capacity	-Low targetability -Poor biodegradability
Nanohydrogel-based	mRNA encapsulated with a hydrogel matrix for delivery	-Good biocompatibility -Stability -High efficiency	-Complex preparation processes -Costly
CNE-based	mRNA formulated with emulsions containing cationic lipids	-well tolerated and immunogenic -good stability	-Particular manufacturing processes -Low targetability

DC: Dendritic cell; LNP: Lipid nanoparticle; CNE: Cationic emulsion

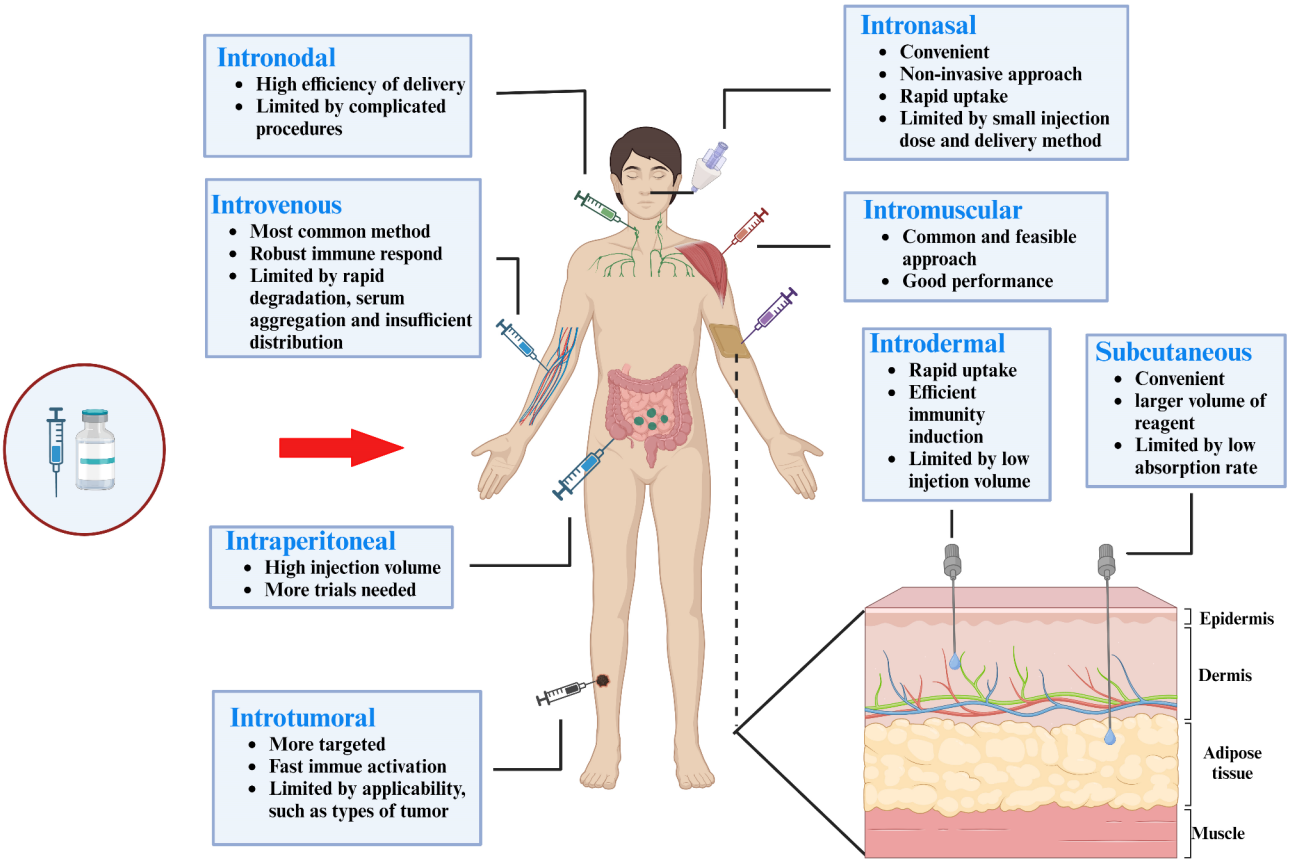

Fig. 5 The common administration routes of mRNA cancer vaccines. The advantages and disadvantages of diverse administration routes for mRNA cancer vaccines were illustrated. Figure created with BioRender (biorender.com)

IM injection is a prevalent vaccination of local delivery with several characteristics, such as easy operation, high immunogenicity, well tolerance, flexible dosing options, as well as minimal side effects at the injection site. Of note, it has been found that this method has specific requirements for particle size and charge, which smaller particle sizes and charges is preferred for delivery and efficacy of mRNA vaccines [127]. The other two common administration routes for mRNA cancer vaccines are ID and SC injections. ID injection can induce a Th1-type immune response effectively due to the high presence of APCs in epidermis and dermis and ideal microenvironment for efficient Ag trafficking, thereby less dose of vaccines is required. However, ID injection is limited by its low injection volume and some local adverse effect (e.g. swelling, pain, erythema, and pruritus) [128]. In contrast, the subcutis region contains less APCs since it is primarily composed of a loose network of adipose tissue. Therefore, a larger volume of reagent can be injected subcutaneously and SC administration can be operated several sites simultaneously. The main disadvantage of SC injection is its low absorption rate, which may result in the degradation of mRNA vaccines [129]. Additionally, intranodal administration and intratumoral injection also provide promising alternatives for mRNA vaccines delivery. Obviously, IN administration means direct injection of mRNA vaccines into lymph nodes, where high amount of local immune cells are available. In line, IN administration has high efficiency of delivery and rapid engulfment of the exogenous mRNA payload by APCs because of close proximity to these cells [130]. Clinical trials using IN injection have been carried out in patients with advanced melanoma and hepatocellular carcinoma (HCC) [131]. In addition to the involvement of complicated procedures, major limitation of IN administration is the transient colocalization of vaccine components at lymph nodes. As a result, the quality and longevity of the vaccine-induced immunological memory may be limited by too quick clearance of the vaccine [132]. Intratumoral injection of mRNA cancer vaccines is also being investigated, since it can offer the advantage of rapid and specific activation of tumor-resident T cells and minimize off-target. Many studies demonstrated that high infiltration of immune cells is associated with higher treatment responses and improved survival. In line with this, direct injection of mRNA cancer vaccines into the tumor may enable a fast activation and expansion of possibly pre-existing antigen-specific T cells, facilitating the switch

from cold tumor to warm tumor. Therefore, the efficacy of IT administration is largely dependent on the immune cell composition within a tumor and the characteristics of surrounding tumor microenvironment (TME) [113, 133]. Currently, IT administration is mainly used for mRNA vaccines encoding immunocostimulatory molecules as immunoadjuvant therapy and repeated injections may be required to maintain the immune response over time. More recently, intranasal immunization was used because of its non-invasive nature, convenience for operation, amenability for repeated administration and high patient compliance. Preclinical studies have shown that abundant APCs and immune cells at nasal mucosa can efficiently induce humoral and cell-mediated immune responses [134]. Moreover, intranasal administration can also deliver mRNA specifically to some tissues, offering alternative routes for targeted delivery [135]. Besides the delivery routes aforementioned, other delivery methods are currently under exploration, such as aerosol inhalation, intraocular injection, intraperitoneal and intracerebroventricular injection [136, 137]. Owing to fewer application scenarios and more challenging operation, the feasibility and practicality of these methods are still under early investigation. Finally, the oral administration of mRNA vaccines should be mentioned. It is well-known that the oral route is one of the first routes for administering therapeutics, including vaccines. In comparison with the parenteral routes aforementioned, oral mRNA vaccines are more preferred due to several inherent advantages, such as self-administration, improved patient compliance, non-invasive and convenience. More importantly, oral administration has the capacity to induce immense immunization, as gastrointestinal tract houses the highest concentration of immune cells in the body [138]. Currently, the main challenges for oral mRNA vaccines are related to the degradation due to unfavorable conditions in gastroenteric tract. Fortunately, recent studies demonstrated that these obstacles may be overcome by proper formulation, such as bacterial extracellular vesicles (EVs) with genetic modification, natural plant-derived EVs or glucans encapsulated within LNPs [139, 140].

Since the distribution of immune cells varies significantly in different tissues, each administration route for mRNA cancer vaccines has unique pros and cons. Moreover, even for the same mRNA vaccine, the administration route can have different effects on the immune response against cancer cells. For example, regarding OVA RNA complexed with DOTAP-DOPE, the killing effects on target cells induced by intravenous administration were superior to those induced by intradermal or subcutaneous administration [141]. While for OVA mRNA combined with TriMix, the in vivo cytotoxicity of antigen-specific T cells induced by intranodal delivery was significantly stronger than that induced by intradermal delivery of the vaccine [142]. Hence, innovative methods and advanced tools are required to monitor and analyze the spatiotemporal kinetics of vaccines accurately in vivo, so that proper guidance and objective evaluation can be offered about the dose and frequency, injection site, and biological distribution of the vaccine. Collectively, the optimization of mRNA cancer vaccination routes is favorable to maximize the efficacy and minimize the side-effect of mRNA vaccines, although it may be hard to predict the best administration route for a specific vaccine accurately.

Clinical trials of mRNA cancer vaccines

Initially, mRNA vaccines were developed as a prophylactic approaches mostly for infectious diseases. Even today, there are about 70% mRNA vaccine trials ongoing remains focused on bacterial and viral diseases. Meanwhile, the remarkable success of COVID-19 vaccines extended the application of mRNA vaccines rapidly to the therapeutic intervention of various incurable diseases, particularly in oncotherapy [143]. In this section, we attempt to make a systemic summary about the clinical trials in different cancer treatments based on the corresponding targets of mRNA cancer vaccines. Moreover, diverse therapeutic regimes (e.g. monotherapy vs. combination therapy) and novel strategy (e.g. personalized mRNA vaccines) were discussed to facilitate the potential application of mRNA cancer vaccines further.

Clinical trials of TAA mRNA vaccines

Due to the preferential expression in tumor cells, TAAs are common used in mRNA vaccine. For instance, several typical TAAs have been identified in melanoma, such as tyrosinase, gp100, MAGE-A3 and MAGE-C2. Generally, TAAs can be divided into three main categories, including cancer germline/testis antigens (e.g. MAGE and NY-ESO-1), cell lineage differentiation antigens (e.g. gp100 and PSA), as well as proteins related to differentiation, proliferation, and anti-apoptosis contributing to the malignant phenotype (e.g. CEA and HER2/Neu). From the first cancer therapy making use of TAA mRNA vaccines in 1995 [18], a series of TAA mRNAs were validated in different mouse cancer models [53, 144]. The success of these preclinical studies paved the way for the initiation of clinical trials (Table 2).

Practically, considering the low immunogenicity induced by the single TAA, the use of mRNAs encoding cocktails of shared TAAs has become the main trend for clinical cancer vaccines. With respect to drug delivery, DC-based platforms account for majority of mRNA cancer vaccines in clinical trials. For hematological malignancy, therapeutic effects of DCs electroporated with diverse mRNAs-encoding products (WT1; WT1/

Table 2 Representative clinical trials of mRNA cancer vaccines encoding TAAs

Sponsor	Name	Antigen	Formulation and Route	Combination	Cancer type	Phases	NCT ID
University Hospital, Antwerp	-	WT1	DC-loaded, i.d.	standard therapy	AML, CML, MM	II	00965224
Medigene AG	-	WT1/ PRAME	DC-loaded, i.d.	-	AML	I/II	02405338
University of Munich	-	WT1/PRAME/ CMVpp65	DC-loaded, i.d.	-	AML	I/II	01734304
Memorial Sloan Kettering Cancer Center	-	WT1/ MAGE-A3/CT7	DC-loaded, i.d.	standard therapy	MM	I	01995708
Steinar Aamdal	-	hTERT/ survivin/ autologous tumor cell mRNA	DC-loaded, i.v.	Temozolomide	Melanoma	I/II	00961844
Bart Neyns	-	MAA	TriMix-DC, i.v./i.d.	-	Melanoma	I	01066390
Inge Marie Svane	-	hTERT/PSA/ PAP/ survivin	DC-loaded, i.d.	Docetaxel	Prostate cancer	II	01446731
University Hospital, Antwerp	-	WT1	DC-loaded, i.d.	-	Solid tumors	I/II	01291420
Oslo University Hospital	-	hTERT/ survivin	DC-loaded, i.d.	Temozolomide	Glioblastoma	II/III	03548571
Steinar Aamdal	-	hTERT/ survivin	DC-loaded, -	platinum	Ovarian Cancer	I/II	01334047
Abramson Cancer Center	-	CD3/ CD28	DC-loaded, i.d.	Bevacizumab	Ovarian cancer	I	01312376
Guangdong 999 Brain Hospital	-	CEA	DC-loaded, -	-	brain metastases	I	02808416
Inge Marie Svane	-	hTERT/ survivin/p53	DC-loaded, i.d.	Cyclophosphamide	Breast Cancer and Melanoma	I	00978913
Affiliated Hospital to Academy of Military Medical Sciences	-	MUC1/ SOCS1/ survivin	DC-loaded, -	CIK cells	NSCLC	I/II	02688686
BioNTech SE	BNT111	NY-ESO-1, MAGE-A3, tyrosinase, TPTE	Lipo-MERIT, i.v.	-	melanoma	I	02410733
BioNTech SE	BNT111	NY-ESO-1, MAGE-A3, tyrosinase, TPTE	Lipo-MERIT, i.v.	Cemiplimab	Melanoma	II	04526899
BioNTech SE	BNT112	PAP, PSA and three undisclosed antigens	Lipo-MERIT, i.v.	Cemiplimab	Prostate cancer	I/II	04382898
BioNTech SE*	BNT113	HPV16 E6 and E7	Naked RNA, i.d.	anti-CD40	$HPV16^+$ cancer	I/II	03418480
BioNTech SE	BNT113	HPV16 E6 and E7	Lipo-MERIT, i.v.	Pembrolizumab	$HPV16^+$ HNSCC	II	04534205
BioNTech SE	BNT114	hTERT/ survivin/p53	Lipo-MERIT, i.v.	-	TNBC	I	02316457
BioNTech SE*	BNT115	3 selectedTAAs	Lipo-MERIT, i.v.	carboplatin/paclitaxel	Ovarian cancer	I	04163094
CureVac*	CV9103	PSA/PSCA/PSMA/STEAP1	RNActive®, i.d.	-	Prostate cancer	I/II	00906243

Table 2 (continued)

Sponsor	Name	Antigen	Formulation and Route	Combination	Cancer type	Phases	NCT ID
CureVac	CV9104	PSA/PSCA/PSMA/STEAP1/PAP/MUC1	RNActive®, i.d.	-	Prostate cancer	II	02140138
CureVac	CV9104	PSA/PSCA/PSMA/STEAP1/PAP/MUC1	RNActive®, i.d	-	Prostate cancer	I/II	01817738
CureVac	CV9201	NY-ESO-1/MAGE-C1/MAGE-C2/survivin/5T4	RNActive®, i.d	-	NSCLC	I/II	00923312
CureVac	CV9202	MUC-1/survivin/5T4/NY-ESO-1/MAGE-C1/MAGE-C2	RNActive®, i.d	-	NSCLC	I	01915524
CureVac	CV9202	MUC-1/survivin/5T4/NY-ESO-1/MAGE-C1/MAGE-C2	RNActive®, i.d	Durvalumab/ Tremelimumab	NSCLC	I/II	03164772

* collaborator

AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; DC: Dendritic cell; MM: Multiple myeloma; HNSCC: Head and neck squamous cell carcinoma; TNBC: Triple negative breast cancer; NSCLC: Non-small cell lung cancer; HPV: Human papillomavirus; CIK cells: Cytokine-induced killer cells; hTERT, Human telomerase reverse transcriptase gene; NY-ESO-1: New York esophageal squamous cell carcinoma 1; MAGE: Melanoma-associated antigen; MUC1, Mucin 1; PAP: Prostate alkaline phosphatase; PRAME, Preferentially expressed antigen in melanoma; PSA, Prostate-specific antigen; PSCA, Prostate stem cell antigen; PSMA, Prostate-specific membrane antigen; STEAP: Six-segment transmembrane epithelial antigen of prostate; TPTE: Trans-membrane phosphatase with tensin homology; WT1, Wilms' tumor 1; 5T4: Trophoblast glycoprotein; i.d.: Intradermal injection; i.v.: Intravenous injection

PRAME; WT1/PRAME/CMVpp65) were evaluated in acute myeloid leukemia (AML). The results showed increased antigen-specific T cells, induced antibody responses, and improved overall survival (OS) [145]. Also, Chung and partners reported that mRNA-electroporated DCs (WT1/MAGE-A3/CT7) is safe and induces antigen-specific immune reactivity together with standard maintenance therapy for multiple myeloma (MM) [19, 130]. In the context of solid tumors, early tests of mRNA-loading DCs mainly focused on melanoma (hTERT/survivin; MAA), prostate cancer (hTERT/PSA/PAP/survivin), and glioblastoma (WT1; hTERT/survivin) [146]. In recent years, multiple clinical trials of mRNA-transfected DC vaccines are conducted for the treatment of various cancers including but not limited to ovarian cancer (hTERT/survivin; CD3/CD28), colorectal cancer (CEA), breast cancer (hTERT/surviving/p53), and NSCLC (MUC1/SOCS1/survivin) [147]. However, most of these registered trials are still under phase I/II, indicating that it is too early to make a decisive evaluation about safety, tolerability, and efficacy of TAA mRNA vaccines loaded by DCs [26].

Apart from DCs, IVT mRNA-based oncotherapies delivered by non-viral vectors are extensively explored as a result of the recent progress in vehicle materials and the promising outcomes from preclinical studies. Many reputable biopharmaceutical companies and research groups devoted to the development of such vaccines and considerable progress have been achieved. As one of pioneers, BioNTech has several well-known candidates for clinical trials, named from BNT111 to BNT115. The BNT111 mRNA, encoding four different TAAs (NY-ESO-1, MAGE-A3, tyrosinase, TPTE), is designed to treat advanced melanoma [148]. In contrast, BNT112 (PAP, PSA and three undisclosed antigens) and BNT115 (which encodes a mixture of three ovarian cancer TAA-encoding RNAs) are designed to treat prostate and ovarian cancers respectively, which are both at Phase I currently. Additional examples include BNT113 (which encodes HPV16-derived tumor antigens E6 and E7) and BNT114 (which encodes a mix of selected breast cancer antigens). Most of these products complex TAA mRNAs with cationic lipid (e.g. DOTMA or DOTAP) and belong to a part of the Lipo-MERIT, a well-known mRNA vaccine project sponsored by BioNTech and collaborators [149]. As another player in the campaign, CureVac has also developed a series of RNActive vaccines that use TAAs-encoding mRNAs and co-delivered with protamine for cancer treatment. The typical candidates include CV9103 and CV9104 for prostate cancer, as well as CV9201 and CV9202 for NSCLS [82]. In the cohort with 48 participants, the application of CV9103 (PSA/PSCA/PSMA/STEAP1) showed well tolerated and prolonged patient survive [150]. Compared with CV9103, CV9104 is a

mixture of six different antigens (PSA/PSCA/PSMA/STEAP1/PAP/MUC1) for prostate cancer encoded by six different mRNAs. The results showed that CV9104 offers a high specificity as only antigen positive tissues are subject to the therapeutic effect [17, 151]. For CV9201, five TAAs (NY-ESO-1/MAGE-C1/MAGE-C2/survivin/5T4) were selected for the immunotherapy of NSCLC through a protamine/RNA delivery system. The results showed that B cells targeting specific antigens have a more than twofold increase in most patients after treatment [152]. Another vaccine candidate developed by CureVac is CV9202, which is composed of IVT mRNAs encoding six different antigens (MUC-1/survivin/5T4/NY-ESO-1/MAGE-C1/MAGE-C2) targeting NSCLC. The results showed that the vaccine treatment was well tolerated and increased antigen-specific immune response was observed in majority of the patients (84%) [107].

Despite of their potential in some clinical trials, it should be noted that the therapeutic effects of monotherapy with TAAs-targeted mRNA vaccines were far from satisfying. The limitations can manifest as low efficiency, poor T cell responses, as well as immune tolerance. To circumvent these limitations, the combinations of mRNA vaccines with conventional treatments like chemotherapy or other immunotherapeutic agents (e.g. ICIs) provided new avenues to augment antitumor efficacy. For instance, a phase II trial was further implemented through the combination of BNT111 with cemiplimab (an anti-PD1 antibody) in advanced stage III/IV melanoma patients unresponsive to anti-PD-1 therapies [148]. BNT113 combined with pembrolizumab versus pembrolizumab alone in patients with HPV16$^+$ head and neck cancer expressing PD-L1 is also under a phase II trial [153]. Similarly, CV9202 has also been evaluated in phase II studies in combination with the durvalumab (an anti-PD-L1 antibody) or the tremelimumab (an anti-CTLA4 antibody). A median progression-free survival of 2 months with the mRNA vaccine+durvalumab alone and 1.8 months with vaccine+durvalumab+tremelimumab were reported [107].

Clinical trials of TSA mRNA vaccines

As described in previous sections, several formidable shortcomings impede the further application of TAA vaccines. TSAs (neoantigens) become more appealing targets for cancer vaccine, because these antigens only present in mutation-induced malignancies, but absent in normal cells. Moreover, the uniqueness of TSAs to individual tumors not only circumvents the issues of tolerance and autoimmunity often associated with targeting shared tumor antigens, but also promotes personalized vaccines as a hotspot in tumor vaccine development. In this context, TSAs-based or personalized cancer vaccines have been designed and examined in the majority of recent clinical trials (Table 3).

Like other types of cancer vaccines, multiple delivery strategies have been developed for TSAs, either through direct injection of unformulated antigens, DC-based autologous transfer, or biomaterial-based delivery system. For instance, DC can be transfected with a neoantigen-encoded mRNA to improve the therapeutic effect. However, based on this approach, there is only

Table 3 Representative clinical trials of mRNA cancer vaccines encoding TSAs

Sponsor	Name	Antigen	Formulation and Route	Combination	Cancer type	Phases	NCT ID
Guangdong 999 Brain Hospital	-	Personalized neoantigens	DC-loaded, i.d.	-	Brain metastases	I	02808416
BioNTech SE	BNT121	10 neoantigens	Naked mRNA, i.n.	-	Melanoma	I	02035956
BioNTech SE*	BNT122	20 epitopes	Lipo-MERIT, i.v.	Atezolizumab	Advanced or metastatic tumors	I	03289962
BioNTech SE*	BNT122	20 epitopes	Lipo-MERIT, i.v.	Pembrolizumab	Melanoma	II	03815058
Moderna	mRNA-4157	Personalized neoantigens	LNP , i.m.	Pembrolizumab	Melanoma	II	03897881
Moderna	mRNA-4157	Personalized neoantigens	LNP , i.m.	Pembrolizumab	Solid tumors	I	03313778
Moderna	mRNA-4157	Personalized neoantigens	LNP , i.m.	Pembrolizumab	Melanoma	III	05933577
Moderna	mRNA-5671	KRAS mutations (G12D, G12V, G13D, and G12C)	LNP , i.m.	Pembrolizumab	NSCLC, CRC, pancreatic cancer	I	03948763

* collaborator

CRC: Colorectal cancer; DC: Dendritic cell; LNP: Lipid nanoparticle; NSCLC: Non-small cell lung cancer; i.n.: Intranodal injection; i.m.: Intramuscular injection

one completed clinical trial to investigate the safety and efficacy in patients with brain metastases and the results is still unavailable [20]. From the first human TSA-based mRNA vaccines were developed and tested in melanoma patients, non-viral vector-based delivery of mRNA vaccines encoding different TSAs has drawn significant attention. The representative iNeST platform has been developed by BioNTech and GeneTech for patient-specific cancer therapy, such as BNT121 and BNT122. The BNT121 vaccine containing 10 neoantigens was examined in 13 melanoma patients by intranodal administration [154]. It was found to induce T cell infiltration to kill tumor cells and to have recurrence-free disease activity. Strong immunogenicity has also been observed in a number of tumor types following injection of BNT122 (Brand name: RO7198457), which contains up to 20 patient-specific novel epitopes. Moreover, the combination of personal mRNA vaccines with other therapeutic methods was also tested in the additional clinical trials [125].

mRNA-4157 and mRNA-5671 are the other two typical personalized mRNA vaccines developed by Moderna and collaborators. mRNA-4157 (Brand name: V940) encoded a repertoire of 34 antigens with strong immunogenicity targeting unique mutations in individual patient from melanoma, NSCLC, and other solid tumors. After intramuscular injection of LNP-encapsulated vaccine, mRNA-4157 showed an acceptable safety profile and observed clinical responses in patients with melanoma and solid tumors. Notably, because of the positive responds of the combinational therapy with mRNA-4157 and pembrolizumab (an anti-PD-1 antibody), mRNA-4157 is the first mRNA cancer vaccine to reach Phase III clinical trials to date [155]. mRNA-5671 (Brand name: V941) was designed to target the four most common cancer-driving KRAS mutations (G12D, G12V, G13D, and G12C) that occur in solid tumors, including NSCLC, colorectal cancer, as well as pancreatic cancer. Currently, mRNA-5671 is in Phase I trials involving 100 patients with diverse solid cancers, in which the safety and tolerability is being assessed as a monotherapy and in combination with pembrolizumab. Results suggested that anti-tumoral immune response was developed and the formulation is overall well-tolerated [143].

Overall, personalized mRNA vaccines based on TSAs (neoantigens) provide a new direction for tumor treatment. It can be anticipated that novel TSAs (neoantigens) with immunogenicity will be predicted and identified with the rapid advancements of next-generation sequencing, bioinformatics approaches and immunologic screening [156]. Although current TSA-based clinical trials are still in their early phases, these encouraging results indicated that personalized mRNA vaccines offer one more promising alternative for neoantigen-abundance cancer treatments, which showed resistant to current immunotherapy, such as immune checkpoint inhibitors.

Clinical trials of mRNA vaccines encoding immunomodulators

Besides TAAs and TSAs, immunomodulators an also be expressed by mRNA to induce APC maturation, activate T-cell mediated immunity and modify the immunosuppressive characteristic of TME, a major obstacle of cancer immunotherapy as mentioned above. To achieve effective translation of immunomodulators-based mRNA vaccines, quite a few companies have made numerous attempts through various delivery platforms, such as DC loading, naked or LNP-formulated synthetic mRNAs [157] (Table 4). From 2008 to 2018, Argos Therapeutics conducted several vaccines in trials using a DC-based platform. Despite the initial trial reported that the combination of cytokine-induced DC maturation with electroporation of CD40L-encoding mRNAs induced elevated IL-12 expression and a robust inflammatory response, the phase 3 trial for the treatment renal cell carcinoma has been terminated due to lack of efficacy [130, 158]. eTheRNA immunotherapies is the other pioneering company in this field. To facilitate DC antigen presentation, TriMix mRNAs encoding CD40, CD40L and the constitutively active TLR4 (caTLR4) were developed and further combine with mRNA coding four TAAs (MAGE-A3/MAGE-C2/tyrosinase/gp100) to make a vaccine product called TriMixDC-MEL. In two autologous DC-based trials (phase II) for melanoma treatment patients with stage III/ IV, TriMixDC-MEL alone or combined with ipilimumab (a CTLA-4 inhibitor) can elicit powerful immune response and result in promising clinical response, such as prolonged disease-free survival rate [159, 160].

In addition to DCs-based platform, naked or non-viral materials-formulated platforms of mRNAs vaccines have also been carried out. One example is ECI-006 from eTheRNA immunotherapies and partners, a combination of TriMix (CD40L, CD70 and caTLR4) and mRNAs encoding five melanoma-specific TAAs (tyrosinase/gp100/MAGE A3/MAGE C2/PRAME), which is at a phase 1 study of melanoma and breast cancer [161, 162]. Similarly, BioNTech, Moderna as well as CureVac also explored several other platforms for cytokine-encoding mRNAs (BioNTech: BNT 151–153 and BNT 131; Moderna: mRNA-2416, mRNA 2752 and MEDI1191; CureVac: CV8102, CV9201 and CV9202) that induce amplified T cell responses and overcome tumor-mediated immunosuppressive effects [163]. First, BioNTech developed several promising mRNA vaccines expressing different immunomodulators. BNT151, a product encoding IL-12, is currently in phase I testing to make a clinical assessment as a monotherapy and in combination with other anti-cancer agents in solid tumors [57].

Table 4 Representative clinical trials of mRNA cancer vaccines encoding immunostimulants and others

Sponsor	Name	Antigen	Formulation and Route	Combination	Cancer type	Phases	NCT ID
Immunostimulants							
Argos Therapeutics	-	CD40L	DC-loaded, i.d.	-	RCC	I/II	00087984
Argos Therapeutics	-	CD40L	DC-loaded, i.d.	-	RCC	III	01582672
Universitair Ziekenhuis Brussel	-	CD40/ CD40L/ MAGE-A3/MAGE-C2/tyrosinase/gp100	TriMix-DC, i.d./ i.v.	-	melanoma	II	01676779
Bart Neyns	-	CD40/ CD40L/ MAGE-A3/MAGE-C2/tyrosinase/gp100	TriMix-DC, i.d./ i.v.	Ipilimumab	Melanoma	II	01302496
eTheRNA	ECI-006	CD40L/ CD70/caTLR4/tyrosinase/gp100/MAGE-A3/MAGE-C2/PRAME	TriMix, i.n.	-	Melanoma	I	03394937
Universitair Ziekenhuis Brussel	-	CD40L/ CD70/caTLR4/tyrosinase/gp100/MAGE-A3/MAGE-C2/PRAME	TriMix, i.t.	-	Breast cancer	I	03788083
BioNTech SE	BNT151	IL-12	lipoplex, i.v.	standard treatment	Solid tumors	I/II	04455620
BioNTech SE	BNT152 + 153	IL-7/ IL-12	lipoplex, i.v.	-	Solid tumors	I	04710043
BioNTech SE*	BNT131	IL-12sc/ IL-15sushi/ IFN-α/ GM-CSF	Saline mixture, i.t.	Cemiplimab/REGN2810	Solid tumors	I	03871348
Moderna	mRNA-2416	OX40L	LNP, i.t.	Durvalumab	Advanced malignancies	I/II	03323398
Moderna	mRNA-2752	OX40L/ IL-23/ IL-36	LNP, i.t.	Durvalumab	Advanced malignancies	I	03739931
Moderna	MEDI1191	IL-12α + IL-12β	LNP, i.t.	Durvalumab	Solid tumors	I	03946800
CureVac	CV8102	TLR7/8,RIG-1	Naked RNA, i.t.	Pembrolizumab	melanoma, SCC and ACC	I	03291002
CureVac	CV8102	TLR7/8,RIG-1	Naked RNA, i.t.	Cyclophosphamide/IMA970A	HCC	I/II	03203005
Others							
BioNTech SE	BNT141	Anti-claudin18.2	mAb, i.v.	nab-paclitaxel/gemcitabine	Solid tumors	I/II	04683939
BioNTech SE	BNT142	CD3/ CLDN6	Naked RNA, i.v.	-	Solid tumors	I/II	05262530

Table 4 (continued)

Sponsor	Name	Antigen	Formulation and Route	Combination	Cancer type	Phases	NCT ID
BioNTech SE	BNT114	p53/ hTERT/ survivin	Lipo-MERIT, i.v.	-	TNBC	I	02316457
Inge Marie Svane	-	p53/ hTERT/ survivin	DC-loaded, i.d.	Cyclophosphamide	Breast Cancer and Melanoma	I	00978913

* collaborator

ACC: Adenoid cystic carcinoma; caTLR4: Constitutively active Toll-like receptor 4; CLDN6: Claudin 6; GM-CSF: Granulocyte-macrophage colony-stimulating factor; HCC: Hepatocellular carcinoma; IFN: Interferon; IL: Interleukin; LNP: Lipid nanoparticle; mAb: Monoclonal antibody; OX40L: OX40 ligand; RIG-1: Retinoic-acid-inducible gene 1; SCC: Squamous cell carcinoma; i.n.: Intranodal injection; i.t.: Intratumoral injection; i.v.: Intravenous injection

A dose escalation trial of BNT152+153 was ongoing to evaluate the safety and anti-tumor activity in patients with solid tumors [147]. Similarly, BNT131 (Brand name: SAR441000) encodes IL-12sc, IL-15sushi, IFN-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) and is under investigation in combination with cemiplimab (an anti-PD1 antibody) via an intratumoral injection in order to alter the tumor microenvironment [164]. Secondly, mRNA-2416 and mRNA 2752, encapsulated in the LNP platform, were developed by Moderna to determine the safety and tolerability of repeated dosing through intratumoral injection. mRNA-2416 encoding OX40L was administered either alone or in combination with durvalumab (a PD-L1 inhibitor) for treatment of lymphoma and metastatic ovarian cancer. Despite the respond to monotherapy is not obvious in 41 patients from a variety of malignancies, a phase 2 cohort in combination with durvalumab for ovarian cancer was launched [21]. mRNA-2752 is a cocktail vaccine consisting of OX40L, IL-23, and IL-36 mRNAs for treatment of lymphoma. In detail, OX40L acts as positive signal to enhance T cell effector function, expansion and survival. IL-36 functions as proinflammatory cytokines to boost anticancer responses and induces a favorable T helper 1 type TME change. IL-23 can play a role as the central coordinators to bridge innate and adaptive immunities [56]. In addition, Moderna and collaborators also developed immunomodulatory fusion proteins named MEDI1191. This agent encodes a single chain fusion protein containing the IL-12α and IL-12β subunits, with a linker between the subunits. Currently, the clinical trial of MEDI1191 in combination with durvalumab is underway to evaluate the effect of intratumoral administration in patients with advanced solid tumors. Additionally, CV8102, the new vaccine against melanoma and other cancers, is currently undergoing or completed phase I testing alone and in combination with anti-PD-1 therapy [165].

Collectively, the delivery of cytokines, co-stimulating ligands, or other immune regulatory factors via mRNA can effectively remodel the TME and enhance tumor sensitivity to various immunotherapies. As a result, tumor vaccines encoding immunostimulants is a promising strategy and have been shown to be effective as adjuvants to tumor immunotherapy to date.

Clinical trials of mRNA vaccines with other strategies

Given the great flexibility of mRNA-based therapeutics, other strategies are also involved in the treatment of cancers apart from aforementioned platforms. Here, due to limited space, we make a brief introduction about mRNA-encoded antibodies, mRNA-based protein replacement therapy and mRNA-encoded antigen receptors (Table 4). Firstly, monoclonal antibodies have been

applied in the field of immune-oncology for a long time. However, traditional antibody manufacturing is hampered by some challenges, including poor cost-efficient production, purification issues, and the need for post-translational modifications. To solve these limitations, mRNA delivery offers an alternative strategy to produce bioactive mAbs and various antibody variants in vivo. Rituximab, targeting CD20, was the first mRNA-encoded mAb approved for cancer therapy [166]. In addition to mAbs, a series of mRNA-encoded bispecific antibodies have been developed. For instance, RiboMabs are bispecific antibody-encoding mRNAs encoding anti-claudin18.2, which can form a bridge between tumor cells and T cells and induces target-dependent T cell activation [167]. In line with this, a product called BNT141 from BioNTech was under exploration. The clinical trial through direct administration of BNT141 showed higher success rates compared to those of chemotherapy [168]. The platform is currently in phase I clinical trials. As a class of bsAbs, bispecific T cell engagers (BiTEs), without the Fc region, consist of two single-chain variable fragments (scFv) joined by a flexible linker. One scFv recognizes the T cell surface protein CD3, whereas the other scFv binds to a target antigen on cancer cells. This specific structure of BiTEs enables the localization of T cells to tumor cells and thus mediates tumor killing [169]. A platform was generated, in which three BiTEs mRNAs target CD3 and one of the three TAAs: claudin 6 (CLDN6), claudin 18.2 (CLDN18.2), or epithelial cell-attached molecules (EpCAM) simultaneously [167]. BNT142, a product of BiTEs (CD3+CLDN6 mRNA) developed by BioNTech, is currently in phase I/II clinical trials [17]. Secondly, protein replacement therapy is an umbrella term aiming to substitute or replenish specific protein deficiencies for medical treatments. Due to the unique advantages, mRNA has been used as a protein replacement platform for prevention and treatment of diseases, in which most are monogenic disorders with dysfunctional proteins, such as factor IX, ornithine transcarbamylase and erythropoietin [21]. As far as cancers concerned, it is well-known that the loss of function of TSGs is usually associated with cancer development, progression, and treatment resistance. Recently, several preclinical studies have shown the feasibility of using mRNAs encoding tumor suppressor genes (e.g., PTEN and p53) to treat tumors. A proof-of-principle study provided in vivo evidence of the restoration of tumor suppression via mRNA-based systemic nanoparticle-mediated delivery of PTEN mRNA [61]. Similarly, Kong and colleagues presented a redox-responsive nanoparticle platform for the effective delivery of TP53 mRNA, which can markedly improve the sensitivity of tumor cells to rapamycin (mTOR) inhibitors for potent combinatorial cancer treatment [170]. Furthermore, mRNAs encoding p53 and tumor antigens (e.g., survivin, hTERT, neoantigens) are currently in clinical trials. Although the application of TSG mRNA has not been extensively explored to date, it can be anticipated that applications of TSG mRNA will be highly attractive for cancer treatment and advance translational medicine in the future. Thirdly, mRNA-encoded CARs or TCRs for T cell engineering represent a promising approach for cancer treatment. The therapeutic potential and safety have been demonstrated in several preclinical and clinical studies. Also, several excellent reviews were recommended here for more details [171, 172].

Current challenges and future outlooks

Although great success of mRNA vaccines in cancer treatment, there are still many tough challenges to be addressed for future application. From the point of the vaccine itself, both intrinsic and external factors can deeply influence the safety, efficiency, as well as clinical applicability and practicality of mRNA cancer vaccines.

The intrinsic factors include selection of antigens with more specificity, structural optimization of mRNA, organ-targeting delivery platforms and proper route of administration [21]. Given the significant impact of the selected antigens on the quality of mRNA vaccines and corresponding immune responses, the screening and identification of TAAs and TSAs (neoantigens) with more specificity and immunogenicity is necessary. Although innovative high-throughput sequencing technologies and novel bioinformatic analyses lead to great facilitation and improvement for these works, relevant experimental validation is usually scant so that the selection of candidate antigens is often subjective and lacks standards [48]. Therefore, it is still a big obstacle for the identification of TAAs and TSAs (neoantigen) with efficiency and reliability through streamlined data mining and experiments. Moreover, the exploration and development of delivery systems with higher targeting capabilities is extremely essential, because targeted therapy not only enables precise killing of tumors but also reduces the side-effects of mRNA vaccines [173]. To solve this problem, some meaningful strategies and related studies have been executed recently. SORT is one of the best-known examples through the modification of the physicochemical properties of delivery materials, such as the structure, molar composition, and charge of the components. In line with this, several organ- or cell-specific targeting, including lymph node, lung and spleen, have been achieved [47]. Another promising strategy for targeted delivery is the surface decoration of vehicles with targeting moieties, such as small-molecule ligands and monoclonal antibodies, to realize specific interact with the target cells. For instance, the selective delivery of mRNAs to CD4+T cells has been successful through the conjugation of

CD4 antibodies to LNPs [174]. Despite of this, achieving targeted mRNA delivery to specific organs is far from satisfactory and remains to be investigated further. Furthermore, as aforementioned, each administration route for mRNA cancer vaccines has unique advantages and limitations. At present, there is no consensus on the optimal route of administration for mRNA vaccines [93]. As a result, it is difficult or even impossible to ascertain the best administration route for a particular vaccine exactly. In this context, direct comparative studies among various routes, such as intramuscular, subcutaneous, intranodal, intradermal, and intravenous vaccination, may be helpful to predict the best administration route for a vaccine.

The external factors mainly involve the tumor heterogeneity and population variations as well as the immunosuppressive TME. Tumor heterogeneity originated from either dynamic evolution of the cancer genomes during tumor progression (temporal heterogeneity) or subclones with different genetic backgrounds (spatial heterogeneity). It is not unexpected that antigen variations resulting from tumor heterogeneity can affect the generation of antitumor T cell responses. For instance, neoantigens, which generated from mutations, can also undergo further mutations leading to the loss or mutation of targeted antigens, which might reduce or even eliminate the therapeutic efficacy of vaccines based on neoantigens. Therefore, the heterogeneity enormously increases the complexity of tumor treatment and is believed to be one of the major causes of vaccine invalid [175]. In addition, it has been demonstrated that some physical conditions, sex, age, and genetic variations could also affect the vaccine's effectiveness. Of note, HLA polymorphisms in different ethnic and regional populations result in differences among individuals in the binding capacity of HLAs to the tumor antigens, and thereby affect the generation and strength of antitumor T cell responses [176]. Additional research must be warranted to elucidate the mechanisms underlying individual differences that will be helpful for the clinical translation mRNA vaccines. Last but not least, effective antitumor responses require the synergistic action of multiple immune cells rather than the action of a single cell. The functions of immune cells depend highly on interactions between the tumor and the associated microenvironment, including surrounding cells, signal molecules, and adjacent extracellular matrix. Accumulating evidences suggested immunosuppressive TME not only promote immune evasion and tumor growth, but also lead to decrease the efficacy of immunotherapy [177]. Therefore, a single administration of vaccine may not be potent enough to overcome the powerful immunosuppressive effects of TME. The combinations of mRNA vaccines with agents that can reverse immunosuppression and block immune checkpoints are more likely to succeed in the treatment of certain cancers [156, 178, 179].

Conclusion

mRNA vaccine attracted widespread attention since the outbreak of the COVID-19 pandemic and garnered international recognition by being awarded the prestigious Nobel Prize in 2023. Indeed, the exciting progress of mRNA vaccine opened a new avenue for the treatment of various diseases, including cancers. Meanwhile, as a relatively new and rapidly evolving technology, mRNA cancer vaccines still face some significant challenges that need to be addressed, such as some practical obstacles like vaccine designs, delivery platforms and administration routes, as well as heterogeneity and immunosuppressive TME leading to extreme complexity of cancers. More in-depth researches and qualified clinical trials will pave the way for promoting this innovative technology to cancer treatment.

Abbreviations

AML	Acute myeloid leukemia
APCs	Antigen-presenting cells
ARCA	Anti-reverse cap analog
bsAbs	Bispecific antibodies
CARs	Chimeric antigen receptors
CARTs	Charge-altering releasable transporters
CDNs	Cyclic dinucleotides
CDS	Coding sequence
CEA	Carcinoembryonic antigen
cmRNAs	Chemically synthesized minimal mRNAs
circRNAs	Circular RNAs
CNEs	Cationic emulsions
COVID-19	Coronavirus disease 2019
CPPs	Cell-penetrating peptides
CTAs	Cancer-testis antigens
CTL	Cytotoxic T-lymphocyte
DCs	Dendritic cells
DODAP	1,2-Dioleoyl-3-dimethylammonium-propane
DODMA	1,2-Dioleyloxy-3- dimethylaminopropane
DOPC	1,2-dioleoyl-sn-glycero-3-ethylphosphocholine
DOPE	1,2-dioleoyl-sn-glycero-3-phosphatidyl-ethanolamine
DOTAP	1,2-dioleoyl-3-trimethylammonium-propane
DOTMA	1,2-di-O-octadecenyl-3-trimethylammonium-propane
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
dsRNA	Double-stranded RNA
HCC	Hepatocellular carcinoma
HPLC	High-pressure liquid chromatography
Hsp70	Heat shock protein 70
ICI	Immune checkpoint inhibitors
IFN I	Type I interferon
ILs	Interleukins
IONPs	Iron oxide nanoparticles
IVT	In vitro transcript
LNP	Lipid nanoparticle
LPX	Lipoplex
mAbs	Monoclonal antibodies
MDA5	Melanoma differentiation-associated gene 5
MHC	Major histocompatibility complex
MM	Multiple myeloma
MYD88	Myeloid differentiation marker 88
NSCLC	Non-small cell lung cancer
ORF	Open reading frame
PAMAM	Polyamidoamine
PAMPs	Pathogen-associated molecular patterns

PBAEs	Polyaminoesters
PEG	Polyethylene glycol
PEI	Polyethyleneimine
PEIM	Preferred iron nanoparticle
PLGA	Poly lactic-co-glycolic acid
PLL	Poly-L-lysine
Pam3	Tri-palmitoyl-S-glycerylcysteine peptide
PRRs	Pattern recognition receptors
RIG-I	Retinoic acid-inducible gene I like receptor
SAM	Self-amplifying mRNA
SORT	Selective organ targeting
ssRNA	Single-stranded RNA
STING	Stimulator of interferon genes
TAAs	Tumor-associated antigens
taRNA	Trans-amplifying RNA
TCRs	T cell receptors
TLRs	Toll-like receptors
TNFs	Tumor necrosis factors
TRIF	Toll-interleukin-1 receptor-domain containing adaptor inducing-IFN-β
TME	Tumor microenvironment
TSAs	Tumor-specific antigens
TSGs	Tumor suppressor genes
UTRs	Untranslated regions
VCE	Vaccinia virus capping enzyme

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Data availability
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Declarations

Patient or public contribution
No patients were involved in this study. The public were not involved in the study design/conduct, the data analysis/interpretation or in the preparation of the manuscript.

Transparency declaration
The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Conflict of interest
All authors have read and approved the final version of the manuscript. The authors declare no conflict of interest.

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