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ENGINEERING MRNA VACCINES FOR SOLID TUMORS: CHALLENGES IN DELIVERY, STABILITY, AND CLINICAL TRANSLATION

Niranjan Giri [1]*, Umesh maske[2], Dr. Kailas Rathod[3]

[1] Master of Pharmacy, Department of Pharmaceutics, Mangaldeep Institute of Pharmacy, Chhatrapati Sambhajinagar, Maharashtra, India
[2] Master of Pharmacy, Department of Pharmaceutics, Mangaldeep Institute of Pharmacy, Chhatrapati Sambhajinagar, Maharashtra, India
[3] PHD, Department of Pharmaceutics, Mangaldeep Institute of Pharmacy, Chhatrapati Sambhajinagar, Maharashtra, India

CORRESPONDING AUTHOR

Niranjan Giri*.
Master of Pharmacy, Department of Pharmaceutics, Mangaldeep Institute of Pharmacy, Chhatrapati Sambhajinagar, Maharashtra, India

Email: sujalchorge@gmail.com

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ABSTRACT

Solid tumors remain a leading cause of cancer death despite advances in surgery, chemotherapy, radiotherapy and immunotherapy. Messenger RNA (mRNA) vaccine technology, validated at scale by the coronavirus disease 2019 (COVID-19) vaccines BNT162b2 and mRNA-1273, offers rapid design, personalization, transient antigen expression and induction of T-cell responses. Applying this platform to solid tumors, however, raises engineering problems that differ from those of infectious-disease vaccines. This review examines three of them: delivery, formulation stability and clinical translation. We discuss lipid nanoparticles (LNPs), polymer-based carriers and hybrid systems, together with sequence and nucleoside engineering and strategies for improving stability and storage. Endosomal escape is a major delivery bottleneck. In studies of LNPs loaded with small interfering RNA (siRNA), only about 1–2% of internalized cargo was found to reach the cytosol in the cell models examined; the corresponding figure for mRNA depends on the formulation, cell type and measurement method and should not be taken as a universal value. Nucleoside modification, notably with N1-methylpseudouridine (m1Ψ), reduces innate immune sensing of in vitro-transcribed mRNA and increases translation, but it does not abolish immune activation, and removal of double-stranded RNA by-products remains important. Clinically, in the phase 2b KEYNOTE-942 trial, adjuvant mRNA-4157 (V940) plus pembrolizumab reduced the risk of recurrence or death by 44% relative to pembrolizumab alone (hazard ratio 0.56 for recurrence-free survival). This is a relative risk reduction in a small, open-label, hypothesis-generating study; it is not a demonstrated improvement in overall survival. Topline phase 3 results announced in August 2026 await full publication, and, to our knowledge, no mRNA cancer vaccine had received regulatory approval at the time of writing. Remaining hurdles include mRNA instability, cold-chain requirements, tumor heterogeneity and immunosuppressive tumor microenvironments.

KEYWORDS mRNA cancer vaccine; lipid nanoparticles; tumor immunotherapy; neoantigen; endosomal escape

1. INTRODUCTION

Solid tumors, which include carcinomas arising from epithelial tissues and sarcomas arising from connective tissues, account for most cancer deaths worldwide. GLOBOCAN 2020 estimated almost 10 million cancer deaths in 2020, with lung, colorectal, liver, stomach and breast cancers contributing the largest numbers [1]. Although surgery, chemotherapy and radiotherapy have improved over several decades, outcomes for tumors such as pancreatic ductal adenocarcinoma (PDAC) and glioblastoma remain poor, which underlines the need for new therapeutic approaches.

Cancer immunotherapy has changed treatment. Immune checkpoint inhibitors that target programmed cell death the nucleus, so it carries no theoretical risk of genomic integration; (ii) can be produced rapidly by cell-free in protein 1 (PD-1), its ligand PD-L1 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have produced durable responses in several cancer types [2]. Many patients, however, do not respond or relapse after an initial response. Contributing factors include a low tumor mutational burden, an immune-excluded or immune-desert phenotype and an immunosuppressive tumor microenvironment (TME) [3,4]. Therapeutic vaccines, which aim to prime or expand tumor-specific T cells, are a complementary strategy.

Messenger RNA has become an attractive vaccine platform. Compared with DNA vaccines and viral vectors, mRNA (i) acts in the cytoplasm and does not need to enter vitro transcription under good manufacturing practice (GMP); (iii) can be redesigned quickly for individual

patients; and (iv) when formulated appropriately, stimulates innate immunity that supports T-cell priming [5,6]. The COVID-19 vaccines BNT162b2 and mRNA-1273 showed about 94–95% efficacy against symptomatic COVID-19 in their pivotal trials [7,8], and this success accelerated the development of mRNA-based cancer vaccines, many of which are now in early- and mid-stage clinical trials.

Moving from infectious disease to cancer brings substantial challenges. Tumor antigens are often weakly immunogenic or subject to immune tolerance, tumors evade immune detection, physical barriers limit nanoparticle penetration, and strong, durable T-cell responses are required. From a pharmaceutical engineering standpoint, three issues stand out: the mRNA molecule is intrinsically fragile; delivery systems must bring the mRNA efficiently into the cytoplasm of antigen-presenting cells; and the properties of the carrier, most commonly an LNP, influence biodistribution, immunogenicity and manufacturability.

This review summarizes the engineering principles behind mRNA vaccines for solid tumors. We cover mRNA design, delivery systems and their limitations, stability and formulation, pharmacokinetics, preclinical and clinical progress, combination strategies, manufacturing and comparison with other vaccine platforms, and we close with future directions. Throughout, we distinguish approved RNA-based therapeutics and approved mRNA vaccines from investigational mRNA cancer vaccines, and we distinguish preliminary findings from established clinical benefit.

2. OVERVIEW OF MRNA VACCINE TECHNOLOGY

2.1 Basic structure of therapeutic mRNA

A therapeutic mRNA molecule comprises five functional elements: a 5′ cap structure (7-methylguanosine, m7G); a 5′ untranslated region (UTR); an open reading frame (ORF) encoding the antigen; a 3′ UTR carrying regulatory sequences; and a poly(A) tail [6,9]. Each element influences translation and stability. The cap is required for ribosome recruitment through eukaryotic initiation factor 4E (eIF4E), and the cap-1 structure (2′-O-methylation of the first transcribed nucleotide) helps the cell to recognize synthetic mRNA as self and is used in current clinical mRNA vaccines [9].

The UTRs strongly affect mRNA half-life and protein yield. Sequences derived from globin genes are widely used because they improve translation and stability [10]. The poly(A) tail, typically 100–150 adenosines in clinical designs, acts together with the cap, through poly(A)-binding protein and eIF4G interactions, to enhance translation and to protect against deadenylation [6,11].

2.2 Types of mRNA platforms

Non-replicating (conventional) mRNA carries only the antigen sequence flanked by regulatory elements. It underlies BNT162b2 and mRNA-1273 and is used in most mRNA cancer vaccines now in clinical testing [12,13]. Its main limitation is transient expression, since the mRNA is degraded within days after delivery.

Self-amplifying RNA (saRNA) encodes an alphavirus-derived replicase so that the RNA can replicate in the cytoplasm after delivery, which may allow lower doses and longer antigen expression [14]. This is potentially useful in cancer, where sustained T-cell stimulation is desirable, and saRNA has entered clinical testing in a neoantigen vaccine [15]. The drawback is size: saRNA constructs are considerably longer than conventional mRNA (typically around 9–12 kb), which complicates manufacturing and formulation.

Circular RNA (circRNA) has covalently closed ends and therefore lacks the free 5′ and 3′ ends that exonucleases attack. Translation is driven by internal ribosome entry site (IRES) elements, and early preclinical work reported more durable protein expression than with linear mRNA [16]. CircRNA remains at the preclinical stage as a vaccine platform.

2.3 Mechanism of action

After intramuscular or intratumoral injection, mRNA–LNPs are taken up by local cells, including muscle cells, dendritic cells (DCs) and macrophages, through endocytic routes such as macropinocytosis and clathrin-mediated endocytosis [17]. In maturing endosomes the pH falls, ionizable lipids become protonated, and the lipids can destabilize the endosomal membrane, allowing a fraction of the mRNA to reach the cytoplasm. Host ribosomes translate the mRNA into antigen. The antigen is degraded by the proteasome and presented on major histocompatibility complex (MHC) class I molecules to CD8+ cytotoxic T lymphocytes (CTLs), or it is secreted or taken up and presented on MHC class II molecules to CD4+ helper T cells [17]. Antigen-bearing DCs migrate to draining lymph nodes, where they activate and expand antigen-specific T cells [12]. Because the antigen is synthesized inside the cell, mRNA vaccines can access the MHC class I pathway more directly than exogenous protein or peptide vaccines (Figure 1).

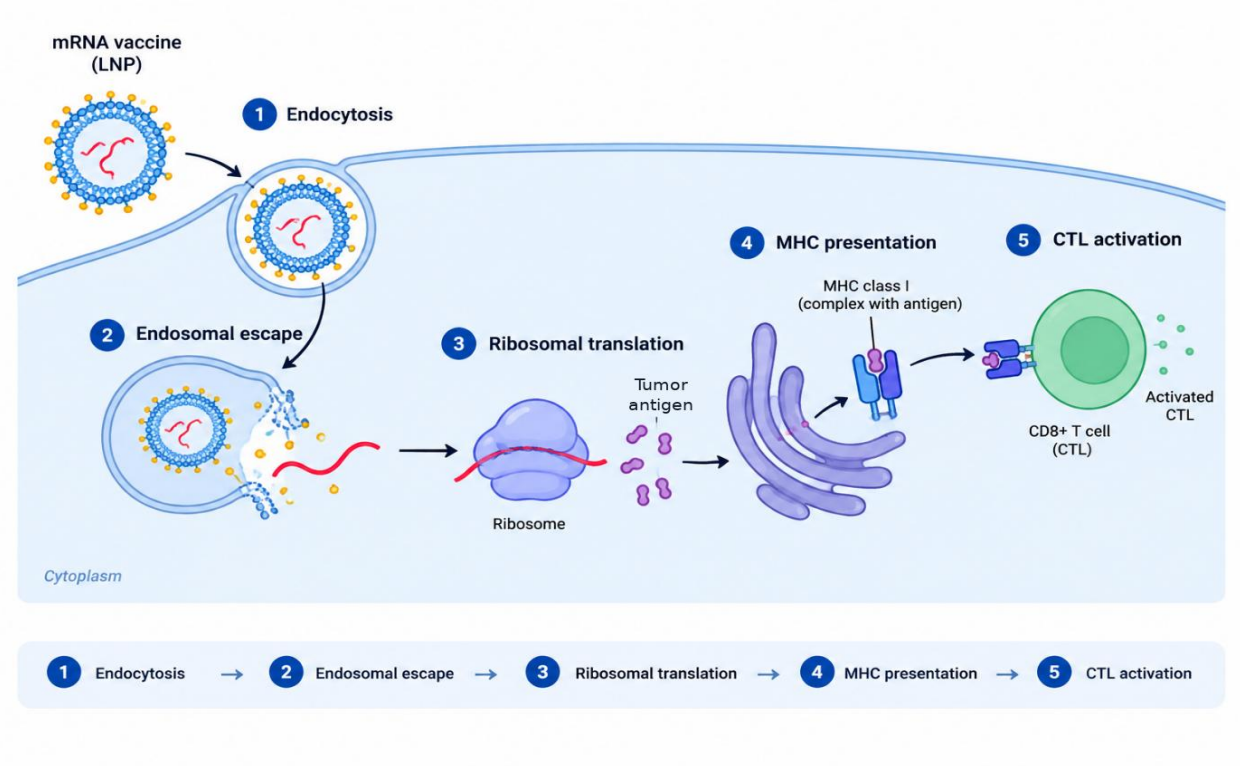


Figure 1. Mechanism of action of mRNA–LNP cancer vaccines: (1) endocytosis; (2) endosomal escape; (3) ribosomal translation of the encoded antigen (a tumor antigen or neoantigen in the cancer setting); (4) MHC class I presentation; (5) CTL activation. MHC class II presentation to CD4+ T cells is not shown. Schematic drawn by the authors.

3. TUMOR BIOLOGY AND IMMUNOLOGICAL CONSIDERATIONS

3.1 Tumor microenvironment

The TME is an ecosystem of cancer cells, stromal cells (fibroblasts, endothelial cells), immune cells and extracellular matrix. In solid tumors it is immunosuppressive and is a main obstacle to vaccine efficacy [3,18]. Key mechanisms include: (i) accumulation of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs); (ii) expression of inhibitory checkpoint molecules, such as PD-L1 on tumor and myeloid cells and CTLA-4 on Tregs and activated T cells; (iii) release of immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β); and (iv) metabolic restriction, including tryptophan catabolism through indoleamine 2,3-dioxygenase (IDO) and lactate accumulation resulting from aerobic glycolysis [3,4].

From a delivery standpoint, solid tumors often have elevated interstitial fluid pressure and dense stroma that impede nanoparticle penetration [19]. The enhanced permeability and retention (EPR) effect, which predicts passive accumulation of nanoparticles in leaky tumor vasculature, is highly variable between tumors and patients, and its value in human tumors is debated [20]. Passive accumulation should therefore not be relied upon as the sole strategy for tumor delivery.

3.2 Tumor antigens

Tumor antigens fall into two broad groups: tumor-associated antigens (TAAs) and tumor-specific antigens, also called neoantigens. TAAs include cancer–testis antigens such as NY-ESO-1 and the MAGE family, overexpressed proteins such as HER2 and MUC1, and differentiation antigens. They are shared between patients, but because they are self-proteins, central and peripheral tolerance often limits the strength of T-cell responses. Neoantigens arise from tumor-specific somatic mutations. They are not subject to central tolerance and can be recognized by high-avidity T cells [21,22]. CD4+ T-cell responses against mutant MHC class II epitopes also contribute to tumor control in preclinical models [23].

Producing a personalized neoantigen mRNA vaccine involves whole-exome sequencing (WES) and RNA sequencing (RNA-seq) of a tumor biopsy and matched normal tissue, computational prediction and prioritization of neoantigens, and GMP manufacture of the patient-specific mRNA [22]. This process currently takes several weeks, and shortening it is a priority for use in the adjuvant setting [22,24].

3.3 Immune evasion and tumor heterogeneity

Tumors can escape vaccine-induced immunity in several ways. They may downregulate MHC class I or lose components of the antigen-processing machinery, such as transporter associated with antigen processing (TAP) and β2-microglobulin; they may lose or alter targeted antigens under immune pressure; and they may drive T cells into exhaustion through chronic antigen exposure [4]. Solid tumors are also heterogeneous in antigen expression across space and time, so a vaccine targeting a limited set of neoantigens may eliminate some tumor cells while leaving behind clones that lack the target. These considerations support vaccines that encode multiple antigens, that include appropriate innate stimulation and that are combined with agents that restore T-cell function.

4. ENGINEERING STRATEGIES FOR MRNA VACCINES

4.1 Sequence optimization

Codon optimization replaces codons with synonymous ones to improve translation in the host cell. Codon composition and secondary structure also influence mRNA stability, so optimization must be tailored to the individual sequence [25]. Excessive optimization can alter folding and translation kinetics and may affect protein conformation. UTR choice also matters: the same coding sequence can give different protein yields with different UTRs, so UTRs should be evaluated for each construct [10].

4.2 Nucleoside modification

Karikó, Weissman and colleagues showed that incorporating modified nucleosides such as pseudouridine (Ψ) and 2-thiouridine into in vitro-transcribed (IVT) RNA reduced activation of Toll-like receptors (TLR3, TLR7 and TLR8) in dendritic cells [26]. Ψ-containing mRNA also showed increased translational capacity and lower activation of the double-stranded RNA-dependent protein kinase (PKR), whose activation suppresses translation [27,28]. N1-methylpseudouridine (m1Ψ), which is used in BNT162b2 and mRNA-1273 [29], gave higher protein expression and lower immunogenicity than Ψ in cell lines and mice [30], and enhanced translation through both eIF2α-dependent and eIF2α-independent mechanisms [31].

These findings support a more limited conclusion than is sometimes stated. Nucleoside modification lowers, but does not abolish, innate sensing of IVT mRNA, and the best-documented effects concern TLR-mediated sensing and PKR activation. The contribution of m1Ψ to evading other cytosolic sensors has been studied less directly. Double-stranded RNA (dsRNA) by-products of IVT are potent triggers of innate responses, and removing them by chromatographic purification, in addition to using modified nucleosides, is needed to obtain mRNA with low immunogenicity and high translational activity [32,33].

4.3 Enhancing translational efficiency

Beyond nucleoside modification, translation can be improved by: (i) co-transcriptional capping with cap-1 analogs, which yields a high proportion of correctly capped mRNA [9]; (ii) optimizing the poly(A) tail, typically 100–150 nucleotides, sometimes interrupted by a short linker [10]; and (iii) removing dsRNA contaminants by purification, which markedly reduces innate activation and increases protein expression [32].

4.4 Modulating immunogenicity

Although modified mRNA reduces innate immune activation, some innate stimulation promotes DC maturation and adaptive immunity, whereas excessive type I interferon signaling can impair antigen-specific responses [34]. The degree of innate stimulation therefore has to be balanced. Approaches include formulation with lipids that have adjuvant properties and co-delivery of immune-stimulating agents or mRNAs encoding cytokines and co-stimulatory molecules [35]. A separate approach, exemplified by the intratumoral programs mRNA-2752 and SAR441000 (BNT131), uses mRNA to express immunomodulatory proteins in the tumor rather than a tumor antigen (Section 9.2); these are not vaccines in the strict sense.

5. DELIVERY SYSTEMS FOR MRNA VACCINES

Unprotected mRNA is rapidly degraded by extracellular RNases, does not cross cell membranes efficiently and can trigger innate immune sensing, so it must be formulated in a delivery system [36]. An effective system must (i) encapsulate and condense the mRNA; (ii) protect it from degradation; (iii) promote uptake by target cells; (iv) enable escape from endosomes; and (v) release the mRNA in the cytoplasm with acceptable tolerability.

5.1 Lipid nanoparticles

LNPs are the most clinically advanced non-viral carriers for RNA. It is important to distinguish between the approved products. Onpattro (patisiran) was the first LNP-formulated RNA therapeutic to be approved (2018); it is an siRNA therapeutic for hereditary transthyretin amyloidosis and is not an mRNA vaccine [37,38]. The approved LNP-formulated mRNA vaccines are BNT162b2 and mRNA-1273, which are directed against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [7,8]. At the time of writing, no mRNA cancer vaccine had been approved (Section 9.2).

Medicinal LNPs contain four components: (1) an ionizable amino lipid, which complexes the mRNA and promotes endosomal escape; (2) a helper phospholipid such as distearoylphosphatidylcholine (DSPC), which supports bilayer structure; (3) cholesterol, which contributes to particle stability and membrane fusion; and (4) a polyethylene glycol (PEG)-lipid, which controls particle size and increases colloidal stability and circulation time [29,39].

The ionizable lipid is the key functional component. It is formulated at acidic pH (around 4), where it is protonated and can complex the negatively charged RNA; it is close to neutral at physiological pH, which reduces toxicity; and it becomes protonated again in the acidic endosome, which promotes membrane destabilization. In siRNA LNPs for hepatic delivery, an apparent lipid pKa of about 6.2–6.5 gave the highest potency [40,41], and similar principles have been applied to mRNA vaccines [42]. Established ionizable lipids include DLin-MC3-DMA (MC3; Onpattro), SM-102 (mRNA-1273) and ALC-0315 (BNT162b2) [29].

Lipid composition and process parameters determine encapsulation, size and in vivo performance. A widely used ionizable lipid:DSPC:cholesterol:PEG-lipid molar ratio is 50:10:38.5:1.5, with nitrogen-to-phosphate (N/P) ratios of about 3–6 and particle diameters of about 80–150 nm [41,42]. Newer LNP designs can alter organ tropism by changing the lipid composition or adding a supplementary lipid, as in selective organ targeting (SORT) LNPs, which can direct mRNA to the spleen, liver or lung [43]. Targeting lymphoid organs is of particular interest for vaccines because they are the sites of T-cell priming [44].

5.2 Polymer-based systems

Cationic polymers, notably polyethylenimine (PEI), form

polyplexes with mRNA, and the 'proton sponge' effect is proposed to aid endosomal release. High charge density, however, is associated with toxicity that has limited the clinical use of PEI [45,46]. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles are biodegradable and well tolerated, but unmodified PLGA particles generally load nucleic acids poorly and escape endosomes inefficiently; this has motivated the addition of cationic or ionizable components [45].

5.3 Lipoplexes and hybrid systems

Conventional cationic liposomes and lipoplexes were among the first mRNA carriers, but toxicity, serum instability and poor targeting limited their use [46]. Nevertheless, a carefully engineered RNA-lipoplex (RNA-LPX) formulation, which delivers RNA to splenic DCs after intravenous injection, is used clinically by BioNTech, for example in BNT111 and BNT122 [47,48]. Lipid–polymer hybrid nanoparticles combine a polymeric core with a lipid shell and have shown activity in preclinical tumor models [49], but their manufacture is more complex than that of LNPs.

5.4 Targeted delivery approaches

Because passive accumulation by the EPR effect is unreliable (Section 3.1), alternative strategies are being explored. These include conjugating targeting ligands that bind tumor-associated receptors to the nanoparticle surface, an approach that has so far been investigated mainly in preclinical studies [44], and intratumoral injection of mRNA encoding immunomodulatory proteins, which bypasses systemic delivery barriers [50].

Table 1. Comparison of major mRNA delivery systems for cancer applications. Values are typical ranges and vary with composition and method.

Delivery system	Typical size	Encapsulation	Clinical use in cancer (examples)	Advantages	Limitations
Ionizable LNPs [29,42]	About 80–150 nm	High	mRNA-4157/V940 (intramuscular); mRNA-2752 (intratumoral)	Potent transfection; clinically validated platform	Liver tropism after intravenous dosing; reactogenicity; anti-PEG antibodies
RNA-lipoplexes [47]	Several hundred nm (formulation-dependent)	High	BNT111, BNT122 (intravenous)	Targets splenic DCs; clinical activity reported	Formulation-specific; manufacturing complexity
Protamine complexes [51]	Formulation-dependent	Moderate	CV9202 (intradermal)	Simple; immunostimulatory	Limited stability; variable potency
PEI polyplexes [45]	Formulation-dependent	Moderate to high	None in advanced trials	High transfection in vitro	Toxicity at effective doses
PLGA nanoparticles [45]	Larger, formulation-dependent	Low to moderate	None in advanced trials	Biodegradable; sustained release	Low loading; poor endosomal escape
Lipid–polymer hybrids [49]	Formulation-dependent	Moderate to high	None in advanced trials	Combine stability of polymer with lipid delivery	Complex manufacturing

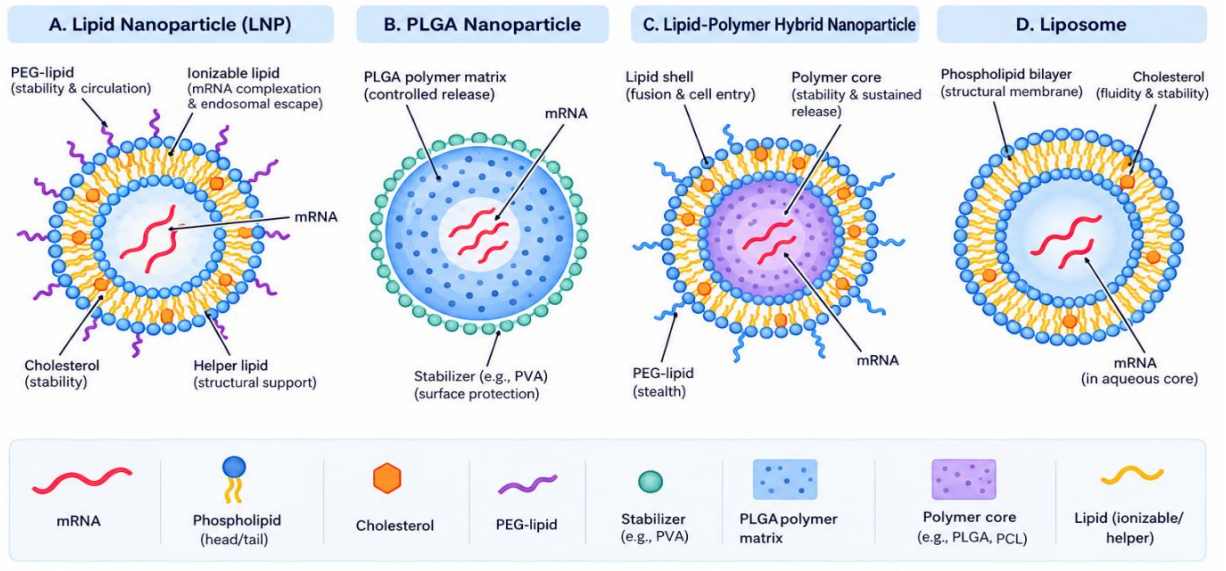


Figure 2. Architecture of mRNA delivery systems: (A) lipid nanoparticle; (B) PLGA nanoparticle; (C) lipid–polymer hybrid nanoparticle; (D) liposome. Schematic drawn by the authors.

6. CHALLENGES IN DELIVERY

Despite advances in LNP engineering, delivery to and within solid tumors remains difficult. Four barriers are discussed below.

Endosomal escape. Escape from the endosome is widely regarded as the rate-limiting step for RNA delivery. In an image-based analysis of siRNA-loaded LNPs, only about 1–2% of the internalized siRNA was released from endosomes into the cytosol, and the release occurred from a limited window during endosomal maturation [52]. A complementary study found that a large fraction of internalized LNP cargo is returned to the extracellular space through endocytic recycling [53]. These figures were obtained for siRNA, with particular LNP formulations, in specific cell models and with particular imaging methods. They should therefore not be treated as universal values for mRNA–LNPs, for which the efficiency may differ with the lipid, the cell type, the dose and the measurement approach [44]. The general conclusion that most internalized cargo fails to reach the cytosol is nevertheless consistent across the field and motivates the development of improved ionizable lipids and of strategies that promote membrane fusion or disruption.

Uptake and biodistribution. After intravenous injection, nanoparticles adsorb serum proteins, which influence their cellular uptake and promote clearance by the mononuclear phagocyte system. For LNPs, adsorption of apolipoprotein E contributes to the strong accumulation in the liver [36,44]. Redirecting particles to tumors or to lymphoid tissue requires changes in lipid composition, PEG-lipid content or targeting moieties, each of which adds complexity to manufacturing [43].

Toxicity and reactogenicity. Cationic and ionizable lipids can cause cytotoxicity and inflammation at high doses [36,39]. PEG-lipids stabilize LNPs but can elicit anti-PEG antibodies, which may accelerate blood clearance of repeated doses, a concern for vaccination schedules that require multiple administrations [39].

The tumor barrier. High interstitial pressure, dense stroma and variable vascular permeability limit penetration of nanoparticles into solid tumors [19,20]. Vaccines that act in lymphoid organs avoid this barrier because they do not need to enter the tumor, whereas intratumoral approaches rely on local injection.

7. STABILITY CHALLENGES

mRNA is a labile molecule, and maintaining its integrity from manufacture to administration is one of the main pharmaceutical challenges. The principal mechanisms are summarized in Table 2.

Enzymatic degradation. RNases are abundant in biological fluids and on surfaces, and unprotected mRNA is rapidly degraded in serum [9,36]. Encapsulation in LNPs shields mRNA from extracellular RNases, but protection is not absolute and depends on the formulation. During manufacture, RNase contamination is controlled with RNase-free reagents and consumables, validated cleaning and controlled production environments; air filtration is a general contamination-control measure for sterile manufacturing and is not a means of preventing RNase-mediated degradation of the product. The integrity of the mRNA in the final product should be confirmed by analytical methods, for example capillary electrophoresis.

Chemical degradation. The 2′-hydroxyl group of ribose makes RNA susceptible to hydrolysis through intramolecular cleavage of the phosphodiester backbone, and the rate depends on pH, temperature and ionic environment [9,54]. Formulation pH must therefore be controlled. A further, formulation-specific pathway has been described in which reactive impurities in ionizable lipids (aldehydes) form covalent adducts with mRNA nucleotides and reduce translation [55]. This underlines the importance of lipid quality control.

Formulation strategies. Lyophilization with cryoprotectants such as sucrose or trehalose is the best-studied route to improving the storage stability of mRNA–LNPs [56,57]. Spray drying and other drying techniques are being investigated. Selection of buffer, pH and excipients is empirical and needs to be optimized for each product [54].

Cold-chain dependency. The licensed COVID-19 mRNA vaccines are stored frozen: the labeled storage condition for BNT162b2 was ultra-low temperature (about −90 to −60 °C) and for mRNA-1273 about −50 to −15 °C, which complicates distribution and increases cost [54]. A lyophilized nucleoside-modified mRNA–LNP influenza vaccine showed no significant change in physicochemical properties or immunogenicity in mice after 12 weeks at room temperature or at least 24 weeks at 4 °C [57]. These are preclinical findings with a model vaccine, and equivalent stability data are needed for the specific formulations used in cancer vaccines, particularly personalized products.

Table 2. Stability challenges and formulation strategies for mRNA–LNP systems.

Stability challenge	Mechanism	Formulation or process strategy	Status and evidence
Enzymatic degradation (RNases)	RNases in serum, on surfaces and in reagents	LNP encapsulation; RNase-free reagents, consumables and controlled manufacturing environment [36]	Encapsulation protects mRNA; degree of protection is formulation-dependent and must be measured by integrity assays
Chemical hydrolysis	2′-OH-mediated backbone cleavage; affected by pH and temperature	pH-controlled buffers; lyophilization; cryoprotectants (sucrose, trehalose) [54,56]	Improves shelf life; conditions are product-specific
Lipid–mRNA adduct formation	Reactive aldehyde impurities in ionizable lipids modify mRNA	Control of lipid purity and impurity profile [55]	Shown for specific lipids; relevant to quality specifications
LNP physical instability	Aggregation or fusion during storage and freeze–thaw	PEG-lipid; optimized lipid ratios; cryoprotectants [56]	Monitored by particle size and polydispersity
Immunostimulatory dsRNA	By-products of in vitro transcription	Chromatographic purification; modified nucleosides [32]	Reduces innate activation and improves translation

Stability challenge	Mechanism	Formulation or process strategy	Status and evidence
Cold-chain dependency	Hydrolysis and LNP changes at higher temperature	Lyophilization [57]	Room-temperature stability shown preclinically for a model vaccine; not yet established for cancer vaccines

8. PHARMACOKINETICS AND BIODISTRIBUTION

The pharmacokinetic (PK) behavior of mRNA–LNPs differs from that of small molecules and conventional biologics. The pharmacologically active species is the antigen produced from the mRNA, so the relationship between dose and response involves a delay and an amplification step [36].

Distribution. After intramuscular injection in rodents, reporter expression is concentrated at the injection site and draining lymph nodes, with detectable expression in the liver depending on the formulation and dose [42]. A fraction of the particles reaches the lymph nodes through lymphatic drainage, where DCs can present the antigen [44]. Quantitative distribution depends strongly on the LNP composition, route and animal model, and it should not be extrapolated directly to patients.

Clearance and duration of expression. mRNA is degraded in the endosomal and cytoplasmic compartments by cellular RNA decay pathways, including decapping and exonucleolytic degradation [58]. In preclinical models, protein expression from conventional mRNA typically peaks within hours to about a day after administration and declines over several days [5,42].

Dose considerations. The dose–response relationship is not linear: cellular uptake and translation are saturable, immune responses do not rise indefinitely with dose, and lipid-related toxicity increases at high doses [39,42]. In clinical neoantigen vaccine trials, dose levels were selected on the basis of safety, tolerability and immunogenicity rather than PK data. For example, in KEYNOTE-942 mRNA-4157 was given intramuscularly at 1 mg every 3 weeks for up to nine doses [59].

9. PRECLINICAL AND CLINICAL PROGRESS

9.1 Preclinical findings

In mouse tumor models, including MC38 colorectal, CT26 colon and B16F10 melanoma, neoantigen-encoding RNA vaccines induced $CD4^+$ and $CD8^+$ T-cell responses and, in some settings, controlled tumor growth [23]. Systemic delivery of RNA-lipoplexes to splenic DCs induced strong T-cell responses and tumor rejection in mice, and the effect was enhanced by checkpoint blockade [47]. Checkpoint blockade has been shown to act on tumor-specific mutant antigens, providing a rationale for combining it with neoantigen vaccines [60]. Intratumoral injection of mRNA encoding cytokines, such as interleukin-12 and granulocyte-macrophage colony-stimulating factor, remodeled the TME and produced antitumor effects in several mouse models [50]. Particle properties, routes of administration and lymphoid-organ targeting all influence the quality of the immune response [43,44].

9.2 Clinical status and trials in solid tumors

Approved products versus investigational cancer vaccines. Three categories should be distinguished. First, RNA-based therapeutics such as Onpattro (patisiran, an siRNA) are approved but are not vaccines [37]. Second, mRNA vaccines against infectious disease, notably the COVID-19 vaccines BNT162b2 and mRNA-1273, are approved [7,8]. Third, mRNA cancer vaccines remain investigational. To our knowledge, none had been approved by a major regulatory authority at the time of writing, although the most advanced program has reported positive topline phase 3 results and regulatory discussions are planned [61]. Table 3 summarizes representative programs.

mRNA-4157/V940 (intismeran autogene). mRNA-4157 is an individualized neoantigen therapy that encodes up to 34 patient-specific neoantigens and is formulated in LNPs. KEYNOTE-942 (NCT03897881) was an open-label, randomized phase 2b study in 157 patients with completely resected high-risk (stage IIIB–IV) cutaneous melanoma, who were assigned 2:1 to mRNA-4157 plus pembrolizumab or pembrolizumab alone; the primary endpoint was recurrence-free survival (RFS) [59]. The hazard ratio for recurrence or death was 0.561 (95% confidence interval 0.309–1.017; two-sided p = 0.053), that is, a 44% relative reduction in the risk of recurrence or death. The 18-month RFS rate was 79% with the combination and 62% with pembrolizumab alone [59]. These data describe recurrence-free survival; the study was not designed or powered to show a difference in overall survival, and overall survival was not shown to improve. Because of the modest sample size, the open-label design and the confidence interval that included 1, the result should be regarded as hypothesis-generating. Grade 3 or higher treatment-related adverse events occurred in 25% of patients receiving the combination and 18% of those receiving pembrolizumab alone [59].

A longer follow-up of KEYNOTE-942 presented at the 2026 American Society of Clinical Oncology Annual Meeting reportedly showed a hazard ratio for recurrence or death of 0.51 (95% confidence interval 0.294–0.887) [62]. In August 2026, the sponsors announced in a press release that the phase 3 INTerpath-001 trial (NCT05933577; 1,137 patients with resected stage IIB–IV melanoma) met its primary endpoint of RFS and a key secondary endpoint of distant metastasis-free survival at a prespecified interim analysis; numerical efficacy results had not been disclosed and the data had not been peer reviewed at the time of writing [61,62]. The program also includes trials in non-small-cell lung cancer (NSCLC), bladder cancer and renal cell carcinoma [61]. The combination has received US Food and Drug Administration (FDA) Breakthrough Therapy designation and European Medicines Agency (EMA) PRIME designation [63].

BNT111. BNT111 is an off-the-shelf mRNA-lipoplex vaccine that encodes four non-mutated melanoma-associated antigens (NY-ESO-1, MAGE-A3, tyrosinase and TPTE). It is formulated as an RNA-lipoplex rather than an LNP. In the phase 1 Lipo-MERIT trial, BNT111 alone or with anti-PD-1 therapy induced antigen-specific T-cell responses in checkpoint-inhibitor-experienced melanoma patients [48]. In the randomized phase 2 trial

BNT111-01 (NCT04526899), 180 patients with anti-PD-(L)1-relapsed or refractory melanoma were randomized to BNT111 plus cemiplimab, BNT111 alone or cemiplimab alone. The objective response rate with the combination was 18.1% (95% CI 10.9–27.4), compared with an assumed historical control of 10% [64]. These are preliminary, conference-reported results from a trial that used a historical control for its primary comparison.

BNT122 (autogene cevumeran). BNT122 is an individualized neoantigen RNA-lipoplex vaccine developed by BioNTech and Genentech. In a phase 1 study in resected PDAC, vaccine-induced neoantigen-specific T cells were detected in a subset of patients [24]. Randomized phase 2 trials are in progress in PDAC (NCT05968326) and in colorectal cancer (NCT04486378). The findings so far concern immunogenicity and feasibility and do not establish clinical efficacy.

Intratumoral immunomodulatory mRNAs. mRNA-2752 (encoding OX40L, IL-23 and IL-36γ; NCT03739931) and mRNA-2416 (encoding OX40L; NCT03323398) are administered intratumorally, and SAR441000 (BNT131; encoding IL-12 single chain, IL-15 sushi, interferon-α and GM-CSF; NCT03871348) is a saline-formulated mRNA mixture, also given intratumorally. These programs encode immune-modulating proteins rather than tumor antigens and are in early-phase testing that focuses on safety and pharmacodynamics. Preclinical rationale is described in [50].

Other programs. CV9202, a protamine-formulated mRNA encoding six NSCLC-associated antigens, was evaluated in a phase Ib study combined with local radiotherapy in stage IV NSCLC [51]. Other early-phase programs include mRNA-5671/V941 (KRAS-mutant tumors, NCT03948763) and the HPV16-directed vaccine BNT113 in head and neck cancer (NCT04534205).

Table 3. Representative clinical programs of mRNA cancer vaccines and related mRNA immunotherapies. Trial identifiers refer to ClinicalTrials.gov records; reported outcomes are limited to what has been published or presented, and preliminary findings are identified as such.

Program (sponsor)	Payload	Formulation; route	Setting	Trial ID; phase	Reported findings and status
mRNA-4157/V940 (Moderna/Merck)	Up to 34 individualized neoantigens	LNP; intramuscular	Resected melanoma; other tumors	NCT03897881, phase 2b; NCT05933577, phase 3	Phase 2b: RFS HR 0.56 (95% CI 0.31–1.02), 44% relative risk reduction; overall survival not shown [59]. Phase 3 topline: primary endpoint met per sponsor; data not yet published [61]
BNT111 (BioNTech)	NY-ESO-1, MAGE-A3, tyrosinase, TPTE	RNA-lipoplex; intravenous	Anti-PD-(L)1-refractory melanoma	NCT04526899, phase 2	ORR 18.1% with cemiplimab vs 10% historical control; preliminary [64]
BNT122/autogene cevumeran (BioNTech/Genentech)	Individualized neoantigens	RNA-lipoplex; intravenous	Resected PDAC; colorectal cancer	NCT03289962, phase 1; NCT05968326 and NCT04486378, phase 2	Phase 1 PDAC: neoantigen-specific T cells in a subset of patients [24]; phase 2 ongoing
BNT113 (BioNTech)	HPV16 antigens	RNA-lipoplex; intravenous	HPV16-positive head and neck cancer	NCT04534205, phase 2/3	Ongoing; no efficacy results cited here
mRNA-5671/V941 (Moderna/Merck)	KRAS mutations	LNP; intramuscular	KRAS-mutant solid tumors	NCT03948763, phase 1	Early-phase; no efficacy results cited here
CV9202 (CureVac)	Six NSCLC antigens	Protamine complex; intradermal	Stage IV NSCLC	Phase Ib with radiotherapy	Phase Ib evaluation reported [51]
mRNA-2752 (Moderna)	OX40L, IL-23, IL-36γ	LNP; intratumoral	Advanced solid tumors, lymphoma	NCT03739931, phase 1	Immunomodulatory mRNA, not a vaccine; early phase
mRNA-2416 (Moderna)	OX40L	LNP; intratumoral	Solid tumors, lymphoma	NCT03323398, phase 1/2	Immunomodulatory mRNA, not a vaccine; early phase
SAR441000/BNT131 (BioNTech/Sanofi)	IL-12sc, IL-15sushi, IFN-α, GM-CSF	Saline (unformulated); intratumoral	Advanced solid tumors	NCT03871348, phase 1	Immunomodulatory mRNA mixture, not a vaccine; early phase

9.3 Safety and tolerability

In KEYNOTE-942, most treatment-related adverse events were grade 1–2, and no mRNA-4157-related grade 4–5 events were reported, although grade 3 or higher treatment-related events were somewhat more frequent with the combination than with pembrolizumab alone [59]. Commonly reported reactions to mRNA vaccines include injection-site pain and systemic symptoms such as fatigue, chills and fever. Because mRNA is translated in the cytoplasm and is not expected to integrate into the genome, integration is not an anticipated risk [5], but long-term safety data for cancer vaccines remain limited, and the safety profile needs to be confirmed in larger trials.

10. COMBINATION STRATEGIES

Immune checkpoint inhibitors. Combining mRNA vaccines with PD-1/PD-L1 or CTLA-4 blockade is a logical strategy: the vaccine increases the number of tumor-specific T cells, and checkpoint blockade relieves inhibition of T-cell function [47,60]. In KEYNOTE-942 the combination was compared with pembrolizumab alone, so the study shows the benefit of adding the vaccine to pembrolizumab but cannot distinguish synergy from an independent vaccine effect, because no vaccine-alone arm was included [59]. The optimal sequence and timing of vaccination relative to checkpoint blockade have not been established.

Chemotherapy. Some cytotoxic agents, such as oxaliplatin and anthracyclines, can induce immunogenic cell death, which involves the release of damage-associated molecular patterns and may enhance priming of anti-tumor T cells [65]. Clinical evidence for combining mRNA cancer vaccines with chemotherapy is limited, although BNT122 is being evaluated together with chemotherapy in PDAC (NCT05968326).

Radiotherapy. Radiation can increase antigen release and inflammatory signals in the tumor and may increase MHC class I expression. The combination of mRNA vaccines with radiotherapy is under clinical evaluation, for example the CV9202 phase Ib study in NSCLC [51]. Abscopal effects (regression of non-irradiated lesions) are uncommon, and robust evidence that vaccines increase their frequency is lacking.

11. MANUFACTURING AND FORMULATION CONSIDERATIONS

Manufacture of a personalized neoantigen mRNA vaccine under GMP comprises (1) sequencing of the tumor and normal tissue (WES and RNA-seq); (2) neoantigen selection and vaccine design; (3) IVT production of the mRNA; and (4) formulation of the mRNA into a delivery system [22]. Critical quality attributes of an LNP product typically include mRNA content and encapsulation efficiency, particle size and polydispersity index, mRNA integrity, residual solvents and process impurities, endotoxin and sterility; specifications are set for each product and agreed with the regulator.

Scale-up. LNPs are usually produced by rapid mixing of a lipid–ethanol phase with an aqueous mRNA phase using microfluidic or impingement mixers, which yields uniform particles [29]. For personalized vaccines, each batch serves a single patient, so GMP production must be flexible, small-scale and fast, which differs from conventional pharmaceutical manufacturing. Automated, integrated platforms that shorten production time are under development.

Regulatory aspects. The regulatory classification of mRNA cancer vaccines depends on the jurisdiction and on the product. In the United States they are regulated as biological products by the Center for Biologics Evaluation and Research of the FDA. In the European Union, mRNA-based cancer immunotherapies may be classified as advanced therapy medicinal products. Regulators expect demonstration of sequence identity, batch consistency, process control and post-marketing safety monitoring. The combination of mRNA-4157 with pembrolizumab has received FDA Breakthrough Therapy designation and EMA PRIME designation [63].

12. COMPARISON WITH OTHER VACCINE PLATFORMS

mRNA versus DNA vaccines. DNA vaccines are more thermostable than mRNA and can give prolonged antigen expression, but the DNA must enter the nucleus, which is inefficient, and DNA vaccines have shown limited immunogenicity in humans compared with results in animals. mRNA acts in the cytoplasm, carries no theoretical risk of genomic integration and has been validated in humans through the COVID-19 vaccines [66].

mRNA versus viral vectors. Viral vectors such as adenovirus and poxvirus are efficient at gene delivery and are intrinsically immunostimulatory. Their use can be limited by pre-existing or induced anti-vector immunity, which may reduce repeated dosing, and personalized vector production is complex. mRNA does not involve a viral vector, so anti-vector immunity is not a concern, although anti-PEG immunity to LNPs may be [22,39].

mRNA versus peptide vaccines. Synthetic peptide vaccines are simple and safe, but they depend on the patient's human leukocyte antigen (HLA) type, and short peptides tend to engage mainly $CD8^+$ T cells; long peptides can engage both $CD4^+$ and $CD8^+$ T cells and have shown immunogenicity in melanoma patients [67]. mRNA vaccines encode the whole antigen, so epitopes presented on both MHC class I and II can be generated without prior HLA typing, because the patient's own antigen-presenting cells select the epitopes [22]. Table 4 summarizes the comparison.

Table 4. Qualitative comparison of mRNA vaccines with other vaccine platforms.

Parameter	mRNA	DNA	Viral vector	Peptide
Nuclear entry required	No	Yes	Vector-dependent	No

Parameter	mRNA	DNA	Viral vector	Peptide
Genomic integration risk	Not expected	Low, theoretical	Vector-dependent (adenoviral vectors do not integrate)	None
Expression duration	Transient (days)	Longer	Variable; can be prolonged	Transient
Anti-vector immunity	Not applicable (anti-PEG antibodies possible)	Not applicable	Can limit efficacy	Not applicable
HLA dependence	No	No	No	Yes
Personalization	Rapid, cell-free manufacture	Feasible	More complex	Feasible
Storage	Frozen or ultra-cold for current licensed vaccines; lyophilization under study	Generally more stable	Frozen or refrigerated, product-dependent	Generally refrigerated or lyophilized
Clinical maturity	Approved for infectious disease; no approved cancer vaccine	Limited human licensure; none for cancer	Approved for some infectious diseases; no approved neoantigen vaccine	Investigational for cancer

13. Limitations and Future Perspectives

The challenges described in Sections 3 and 6–8 define the main limitations of current approaches: inefficient cytosolic delivery, heterogeneous and immunosuppressive tumors, limited stability and cold-chain requirements, and the cost and complexity of personalized manufacture. Several developments may address them.

Artificial intelligence and personalization. Machine-learning models are already used to predict peptide–MHC presentation and are being extended to T-cell receptor recognition and immunogenicity [68]. Similar approaches may help optimize mRNA sequences, for example codon usage, UTRs and secondary structure. Monitoring of circulating tumor DNA could eventually guide updating of vaccine antigens as tumors evolve, although this concept remains to be tested.

Next-generation carriers. Active areas include biodegradable ionizable lipids with improved tolerability [69], organ-selective LNPs such as SORT [43], stimulus-responsive particles and co-delivery of antigen and adjuvant mRNAs. Non-lipid carriers (polymeric systems, cell-derived vesicles) are being explored as alternatives, in part because they may avoid anti-PEG immunity; these remain at an earlier stage than LNPs.

Clinical translation. Key priorities are shortening the time to produce individualized vaccines, developing thermostable formulations, identifying predictive biomarkers (for example tumor mutational burden, neoantigen clonality and HLA diversity), using adaptive trial designs to detect combination signals and assessing cost-effectiveness. The reported phase 3 topline results will, if confirmed in full publications, be an important test of the concept.

14. CONCLUSION

Engineering mRNA vaccines for solid tumors is an active field of pharmaceutical science. Nucleoside modification and the development of LNP delivery systems enabled the COVID-19 vaccines and provided the technology base for cancer vaccines. In the randomized phase 2b KEYNOTE-942 trial, adding mRNA-4157 to pembrolizumab reduced the risk of recurrence or death by 44% in resected high-risk melanoma. This is a relative reduction in recurrence-free survival events from a hypothesis-generating study, not a demonstration of improved overall survival. Topline phase 3 results have been announced but not yet published, and no mRNA cancer vaccine has, to our knowledge, been approved.

Substantial obstacles remain. Only a small fraction of internalized cargo is thought to escape endosomes in the systems studied, mRNA is chemically and enzymatically labile, ultra-cold storage constrains distribution, tumors are heterogeneous and immunosuppressive, and personalized manufacture is complex. Progress will require advances in lipid and carrier design, formulation science, computational antigen selection and rational combination therapy. Whether these advances will make mRNA cancer vaccines part of standard care across solid tumors will depend on the outcome of confirmatory trials.

15. ABBREVIATIONS

Table 5. List of abbreviations.

Abbreviation	Full form
CI	confidence interval
circRNA	circular RNA
CTL	cytotoxic T lymphocyte

Abbreviation	Full form
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
DC	dendritic cell
dsRNA	double-stranded RNA
EMA	European Medicines Agency
EPR	enhanced permeability and retention
FDA	US Food and Drug Administration
GMP	good manufacturing practice
GM-CSF	granulocyte-macrophage colony-stimulating factor
HLA	human leukocyte antigen
HR	hazard ratio
IL	interleukin
IVT	in vitro transcription
LNP	lipid nanoparticle
m1Ψ	N1-methylpseudouridine
MHC	major histocompatibility complex
N/P	nitrogen-to-phosphate ratio
NSCLC	non-small-cell lung cancer
ORF	open reading frame
ORR	objective response rate
PD-1/PD-L1	programmed cell death protein 1/its ligand
PDAC	pancreatic ductal adenocarcinoma
PEG	polyethylene glycol
PEI	polyethylenimine
PKR	double-stranded RNA-dependent protein kinase
PLGA	poly(lactic-co-glycolic acid)
RFS	recurrence-free survival
RNA-LPX	RNA-lipoplex
saRNA	self-amplifying RNA
siRNA	small interfering RNA
SORT	selective organ targeting
TLR	Toll-like receptor
TME	tumor microenvironment
UTR	untranslated region
WES	whole-exome sequencing

16. DECLARATIONS

16.1. Ethical approval and consent to participate

Not applicable.

16.2. Conflict of interest

No potential conflict of interest was reported by the authors.

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16.4. Author's contributions

Investigation, Visualization, Writing - Original Draft: NG, UM. Conceptualization, Project Administration, Validation, Review and Editing: KR. All authors read and approved the final version of the manuscript.

16.5. Artificial intelligence statement

The authors used an artificial intelligence (AI)-assisted language tools to improve the readability and language of the manuscript, as well as to format the tables and generate the illustrative figure. All scientific content, study design, data collection, data analysis, interpretation of the findings, and final editorial decisions were performed entirely by the authors. The authors have

reviewed and approved the final manuscript and accept full responsibility for their accuracy and integrity.

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