Asian J Biol Life Sci., 2027; XX(XX):1-14.
https://ajbls.com

Review Article

Engineering mRNA Vaccines for Solid Tumors: Challenges in Delivery, Stability, and Clinical Translation

Niranjan Giri*, Umesh Maske

Department of Pharmaceutics, Mangaldeep Institute of Pharmacy, Sambhajinagar, Maharashtra, INDIA.

ABSTRACT

Solid tumors are still the cause of cancer deaths around the world even though traditional treatments have improved. Messenger RNA or mRNA vaccine technology has become a change after the success of vaccines for coronavirus disease 2019. This technology allows for personalization, short-term protein production and strong immune responses. Using mRNA vaccines for cancer solid tumors is not the same as for infectious diseases. It comes with its set of challenges that need pharmaceutical engineering to solve. This review looks at the engineering ideas behind developing mRNA vaccines for treating tumors. The focus is on three areas: how to deliver the mRNA, how to keep the formulation stable and what stops the therapy from working in patients. We go over delivery systems, including lipid nanoparticles, polymer-based carriers and mixed or hybrid systems. One important finding is that only a tiny fraction of the mRNA reaches the cytoplasm of the cell about 1 to 2%. This poor endosomal escape is the problem holding back how well these vaccines can work in patients. Also modifying the nucleosides in the mRNA especially using N1-methyl pseudouridine helps reduce reactions while making the mRNA more efficient at producing proteins. Clinical trials show progress. For e.g - the mRNA-4157/V940 vaccine, used alongside a checkpoint inhibitor that targets the PD-1 pathway improved survival by 44% in melanoma patients. This is the controlled trial to show a real benefit from an mRNA cancer vaccine. Still many hurdles remain. The mRNA can break down quickly due to chemical or enzyme activity. Most formulations need storage, which makes them hard to use in many places. Tumors are also very different from one patient, to another. The tumor environment often suppresses the system. All these factors make it hard to move mRNA vaccines into use.

Keywords: Endosomal escape, Lipid nanoparticles, mRNA cancer vaccine, Neoantigen, Tumor immunotherapy.

Correspondence:
Dr. Niranjan Giri
Department of Pharmaceutics,
Mangaldeep Institute of Pharmacy,
Sambhajinagar, Maharashtra, INDIA.
Email:

INTRODUCTION

Solid tumors include cancers like carcinomas and sarcomas which come from tissues and connective tissues. These types of cancer are still the cause of death from cancer around the world (Pardi *et al*., 2018). Data from GLOBOCAN 2020 shows that about 10 million people died from cancer globally with lung, colorectal, liver, stomach and breast cancers making up most of those deaths (Karikó *et al*., 2005). Even though surgical methods, chemotherapy and radiation therapy have improved over the 40 years survival rates for many solid tumors remain very low. This is especially true for ductal adenocarcinoma (PDAC) glioblastoma multiforme and metastatic melanoma. The poor outcomes highlight how urgent it is to find ways to treat these cancers.

Cancer immunotherapy has changed how we approach treatment. Drugs called checkpoint inhibitors, which target PD-1, PD-L1 and CTLA-4 have helped some patients achieve lasting responses across several cancer types (Karikó *et al*., 2008). Many patients do not respond at all or eventually stop responding. This lack of response often comes down to numbers of mutations in the tumor an immune-free environment or a strong immune-suppressing setting inside the Tumor Microenvironment (TME) (Sahin *et al*., 2017). Another way to fight cancerusing vaccines that teach the system to recognize and attack tumor cells offers a different strategy. These active immunotherapies aim to boost tumor- T cells before the disease progresses.

Messenger RNA or mRNA has become a platform for vaccine development. Compared to DNA vaccines and viral vectors mRNA offers benefits: (i) it works only in the cytoplasm and does not integrate into the host genome reducing safety risks; (ii) it can be made quickly and scaled up using good manufacturing practices; (iii) its sequence can easily be customized for individual

DOI: 10.5530/ajbls.20270030

Copyright Information :
Copyright Author (s) 2027 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

patients; and (iv) when properly formulated it naturally activates the immune system (Rojas *et al*., 2023). The success of the BNT162b2 and mRNA-1273 vaccines against SARS-CoV-2 proved that mRNA technology is both effective and safe showing than 90% protection and excellent safety records (Kulkarni *et al*., 2021). Thanks to this progress researchers have moved fast to develop mRNA-based cancer vaccines. Phase clinical trials in solid tumors are now showing encouraging results.

Moving mRNA vaccines from fighting viruses to treating cancer brings big challenges. Solid tumors are tough to beat. They have antigens can change their behavior to avoid immune detection, block delivery of drugs through physical barriers and require strong lasting T-cell reactions against weakly visible tumor proteins. From a standpoint three main issues stand out: mRNA molecules themselves are fragile and unstable; delivery systems must efficiently get the mRNA into target cells; and the properties of Lipid Nanoparticles (LNPs) which deliver mRNAaffect where the particles go in the body how they trigger immune responses and how easy they are to produce.

This review gives a look at the science behind engineering mRNA vaccines for solid tumors. We analyze delivery systems, lipid nanoparticle design as well as polymer-based carriers and hybrid systems. We also explore how mRNA sequences can be optimized discuss stability problems and how to fix them and look at data, from lab studies and human trials. Finally, we identify limitations and point toward future steps that will help shape the next generation of mRNA cancer vaccines.

Overview of mRNA Vaccine Technology

Basic Structure of mRNA

A therapeutic mRNA molecule is made of five parts: (1) a 5′ 7-Methylguanosine (m7G) cap; (2) a 5′ Untranslated Region (UTR); (3) an Open Reading Frame (ORF) that codes for the antigen; (4) a 3′ UTR with regulatory sequences; and (5) a poly(A) tail (Hou *et al*., 2021). Each part is vital for translation and for keeping mRNA stable. The cap part is needed for ribosome recruitment through eIF4E binding and the cap-1 form (2′-O-methylation of the nucleotide) is now used in all clinical mRNA products to lower innate immune response (Miao *et al*., 2021). I find it clear that the cap is the thing the cell notices.

The 5′ and 3′ UTRs are factors that decide how long mRNA lasts and how much protein is produced. Sequences taken from globin genes (α- and β-globin) are often used because they help ribosomes load better and guard mRNA against exonuclease breakdown (Holtkamp *et al*., 2006; Mauger *et al*., 2019). The poly(A) tail, 100-150 adenosine units in clinical designs works together with the 5′ cap to form a loop in mRNA through PABP-eIF4G contacts boosting translation and protecting against deadenylation (Verbeke *et al*., 2021). I think this partnership is a trick the cell uses.

Types of mRNA Platforms

Non-amplifying or conventional mRNA is the design. It carries the antigen of interest surrounded by regulatory UTRs. This design underlies BNT162b2, mRNA-1273 and all current clinical mRNA cancer vaccines (Weng *et al*., 2020). Its main drawback is that expression is short-lived, because mRNA is broken down within days to weeks after it is delivered. I note that this limits how long the immune system can see the antigen.

Self-amplifying RNA (saRNA) takes the replicase system from alphaviruses so that after an initial dose the antigen RNA can replicate inside the cell (Liu *et al*., 2023). This design could save dose. Keep antigen present for a longer time, which might be very useful in cancer, where long T-cell stimulation is needed. saRNA is much bigger (9-12 kb compared with about 1-2 kb for conventional mRNA) which creates extra formulation problems. I see that the size is a trade-off.

Circular RNA (circRNA) is a platform that resists nucleases because its ends are closed together so there are no free 5′ and 3′ ends for exonucleases to attack. Its translation uses Internal Ribosome Entry Site (IRES) elements and early preclinical work shows that it can keep producing protein than linear RNA (Awasthi *et al*., 2022). This makes circRNA a promising option for longer-lasting expression.

Mechanism of Action

After injection into muscle or a tumor mRNA-LNPs are taken up by recruited cells, such as muscle cells, dendritic cells and macrophages using macropinocytosis and clathrin-mediated endocytosis. In the endosome the ionizable lipids become protonated because of the pH (5.0-6.5). This helps the lipid mix with the membrane and lets the mRNA escape into the cytoplasm. In the cytoplasm host ribosomes translate the mRNA into antigen protein. The protein is then broken down by the proteasome. Shown on MHC class I molecules to CD8+ cytotoxic T lymphocytes or it is secreted or taken up for MHC class II presentation to CD4+ helper T cells (Hanahan, 2022). I observe that the cell's own machinery does the lifting here.

Antigen-loaded dendritic cells travel to lymph nodes where matching T cells are activated and multiplied (Binnewies *et al*., 2018). The resulting broad CTL reaction creates memory cells that can spot and kill tumor cells that display the encoded antigen. Importantly when the antigen is made inside the cell from mRNA it mimics a viral infection better than peptides given from outside. This gives two benefits: cross-presentation, on MHC class I and stronger CTL activation (Figure 1).

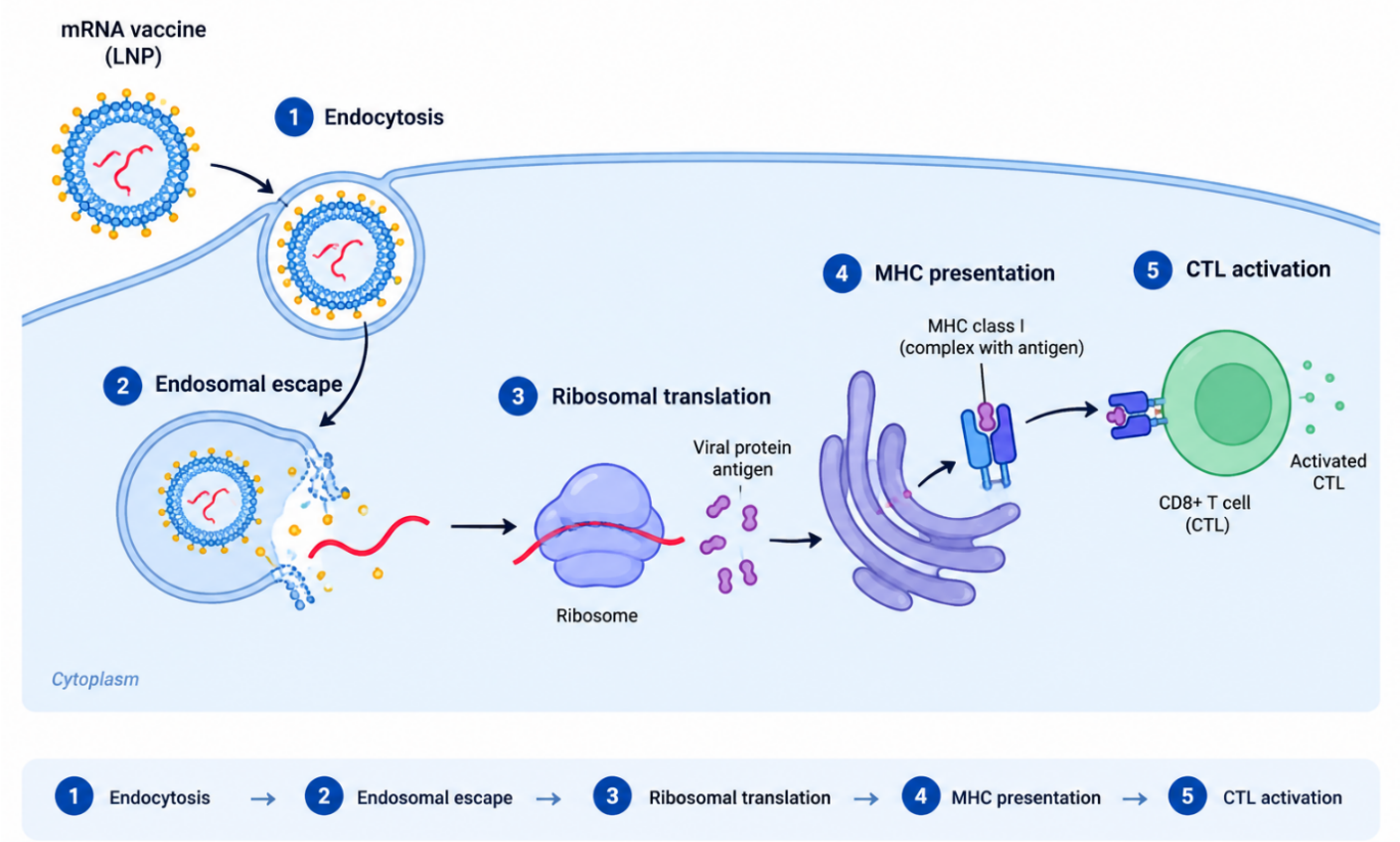


Figure 1: Mechanism of Action of mRNA-LNP Cancer Vaccines.

Tumor Biology and Immunological Considerations

Tumor Microenvironment

The Tumor Microenvironment (TME) is an ecosystem made of cancer cells stromal cells such as fibroblasts and endothelial cells immune cells and an extracellular matrix that contains many inhibitors. In solid tumors the TME suppresses the immune system and is the main obstacle to successful cancer vaccine treatment. Key ways that the TME suppresses the system are:

(i) buildup of regulatory T cells (Tregs) and Myeloid-Derived Suppressor Cells (MDSCs)

(ii) production of immune-checkpoint ligands such as PD-L1 and CTLA-4 by tumor cells and stromal cells

(iii) release of immune-suppressing cytokines such as IL-10 and TGF-β

(iv) loss of nutrients caused by tryptophan breakdown (the IDO pathway) and by lactate build-up from Warburg metabolism.

From a delivery standpoint solid tumors often have Interstitial Fluid Pressure (IFP) that can be 10-40 mmHg higher than normal tissue. This high pressure greatly hinders the movement of nanoparticles and large molecules through the blood vessels in the tumor. Although the Permeability and Retention (EPR) effect suggests that nanoparticles smaller than about 200 nm should build up in tumors because the blood vessels are leaky doctors now doubt how useful this effect is in real patients because blood vessel leakage varies a lot between patients and between different tumors (Chen and Mellman, 2013; Hegde and Chen, 2020; Sabado and Bhardwaj, 2015).

Tumor Antigens

Tumor antigens fall into two types: Tumor-Associated Antigens (TAAs), tumor-specific antigens (TSAs) also called neoantigens. TAAs include cancer-testis antigens such as NY-ESO-1 and the MAGE family, proteins that are found in amounts such as HER2 and MUC1 and antigens linked to cell differentiation. These TAAs are useful for patients but the immune system is used to them so vaccines that target them often cannot produce strong T-cell responses. Neoantigens come from mutations in the tumor. Because the body has never seen them, they are not ignored by the system and can trigger T-cells that bind very tightly.

To make a neoantigen-based mRNA vaccine doctors first perform sequencing (WES) and RNA sequencing (RNA-seq) on a tumor biopsy. Then they use computer analysis to predict neoantigens and score how well they might provoke a response. Finally, they quickly produce the mRNA in a Good-Manufacturing-Practice

(GMP) lab. This whole process can now be finished in four to six weeks.

Immune Evasion Mechanisms

tumors have developed several ways to avoid the immune response that a vaccine would create. They can lower the amount of MHC class I on their surface, which happens in about 40-80 % of cancers. They can also lose parts of the machinery that shows antigens to the system, such as TAP1, TAP2 and β2-microglobulin. Tumors may also. Change antigens when the immune system attacks them. Finally, tumors can push T-cells into a state called exhaustion because the same antigens keep appearing. Because of all these tricks vaccines need to use different antigens have built-in adjuvants and work together with drugs that bring the immune system back, to life (Wesselhoeft *et al*., 2019).

Engineering Strategies for mRNA Vaccines

Sequence Optimization

Codon optimization is the process of replacing codons to make the message work better in the host cell. Replacing with codons that match the used human tRNAs makes the cell work faster and make more protein (Mahase, 2023). It is very important that this is done for the specific protein being made; doing it much can break the way the mRNA folds, which can make the protein not work as well and not be recognized by the immune system.

Good UTR sequences are also very important. Studies that compared beta-globin, alpha-globin, heat shock protein (HSP70) and made UTR sequences showed that the same coding part of the message made different amounts of protein. This shows how important it is to check UTR sequences when making a vaccine (Pollard *et al*., 2013).

Nucleoside Modifications

A big discovery by Karikó and Weissman showed that adding pseudouridine (ψ) and 2-thiouridine (s2U) into mRNA made the body's immune system less active and helped the cell make protein. Later N1-methyl pseudouridine (m1ψ) was found to be the change balancing being hidden from the immune system and making more protein (Svitkin *et al*., 2017). M1ψ stops the body from recognizing the mRNA with pattern recognition receptors like TLR3, TLR7, TLR8 and RIG-I. Lowers the activation of the double-stranded RNA (dsRNA)-dependent Protein Kinase (PKR) which stops the cell from making protein (Semple *et al*., 2010).

Translational Efficiency Enhancement

In addition to changing the nucleosides the process of making protein from mRNA is made better by: (i) making cap structures. Cleancap trinucleotides that make real cap-1 structures work better than old ones by 10-15% in making protein; (ii) making better poly(A) tails 120-150 nucleotides long tails (with short sections of poly(A) in between) help the cell bind and keep the message longer; and (iii) removing double-stranded RNA that is made during the process through HPLC cleaning, which makes the body less active and makes protein better by more than 2 times (Jayaraman *et al*., 2012).

Immunogenicity Modulation

Even though modified mRNA helps the body not react much a little bit of immune reaction is good for the body's long-term protection. This is usually done by mixing with parts that help the system like some lipids or by giving TLR activators together (Maier *et al*., 2013). The new idea is to keep the message that makes the protein (maximally changed for making more) separate from the part that helps the system (given as a separate part or as proteins like GM-CSF, IL-12 or OX40L) as seen in the mRNA-2752 and BNT131 clinical programs.

Delivery Systems for mRNA Vaccines

Uncovered mRNA is quickly broken down by the body's enzymes (life less than 1 min) can't enter the cell and causes immune reactions so it must be put into a protective delivery container (Verbeke *et al*., 2021; Dowdy, 2017). The delivery system needs to: (i) put the mRNA into a capsule using electricity or oils; (ii) protect from being broken down by the body; (iii) get into the cell; (iv) get out of the cells bubble; and (v) release the message in the cell without making the cell sick (Table 1 and Figure 2).

Lipid Nanoparticles (LNPs)

LNPs are the way to deliver mRNA and are the only system that has medicines that are approved by regulators (Onpattro [siRNA] BNT162b2, mRNA-1273). LNPs used in medicine have four parts: (1) a lipid that can change charge which helps the mRNA get inside and escape the cells bubble; (2) a helper lipid (DSPC or DOPE) which keeps the structure stable and helps the cell take it in; (3) cholesterol or a similar part, which helps the shape and how it works with the cell; and (4) a PEG part, which helps the system stay in the body and not be removed too quickly (Hassett *et al*., 2019).

The lipid that can change charge is the important part as its pH level decides how well the mRNA is put in and how it escapes from the cell. The best pH is around 4, where the lipid's positive. Then at the pH of the cells bubble (5-6) the lipid becomes positive again. Breaks the bubble. A big study by Akinc *et al*. (2019). Found that lipids with the pH levels of 6.2-6.5 work best at making genes

Table 1: Comparison of Major mRNA Delivery Systems for Cancer Vaccine Applications.

Delivery System	Particle Size	Encapsulation Efficiency	Route	Advantages	Limitations
LNPs (ionizable)	~80-200 nm	High (>90%)	Systemic/IM/IT	Potent transfection; clinical-grade	Rapid clearance; off-target; toxicity at high dose
Cationic Lipoplexes	~150-500 nm	Moderate (60-75%)	Local/IT	Simple preparation	High toxicity; serum instability
PLGA NPs	~200-600 nm	Moderate (40-70%)	Local/IT	Biodegradable; sustained release	Low endosomal escape; low encapsulation
Polyethylenimine (PEI)	~100-300 nm	Moderate (~60%)	Local/IV	High transfection *in vitro*	High toxicity *in vivo*; immunogenicity
Lipid-Polymer Hybrids	~100-250 nm	High (75-90%)	Systemic/IT	Combined benefits; stability	Complex manufacturing; cost
Protamine Complexes	~50-200 nm	Moderate (~55%)	Intradermal/IM	Low cost; immunostimulatory	Variable encapsulation; aggregation risk
Exosome-based	~30-150 nm	Low-Moderate (~30%)	IV/IT	Natural targeting; low toxicity	Scale-up challenge; batch variability

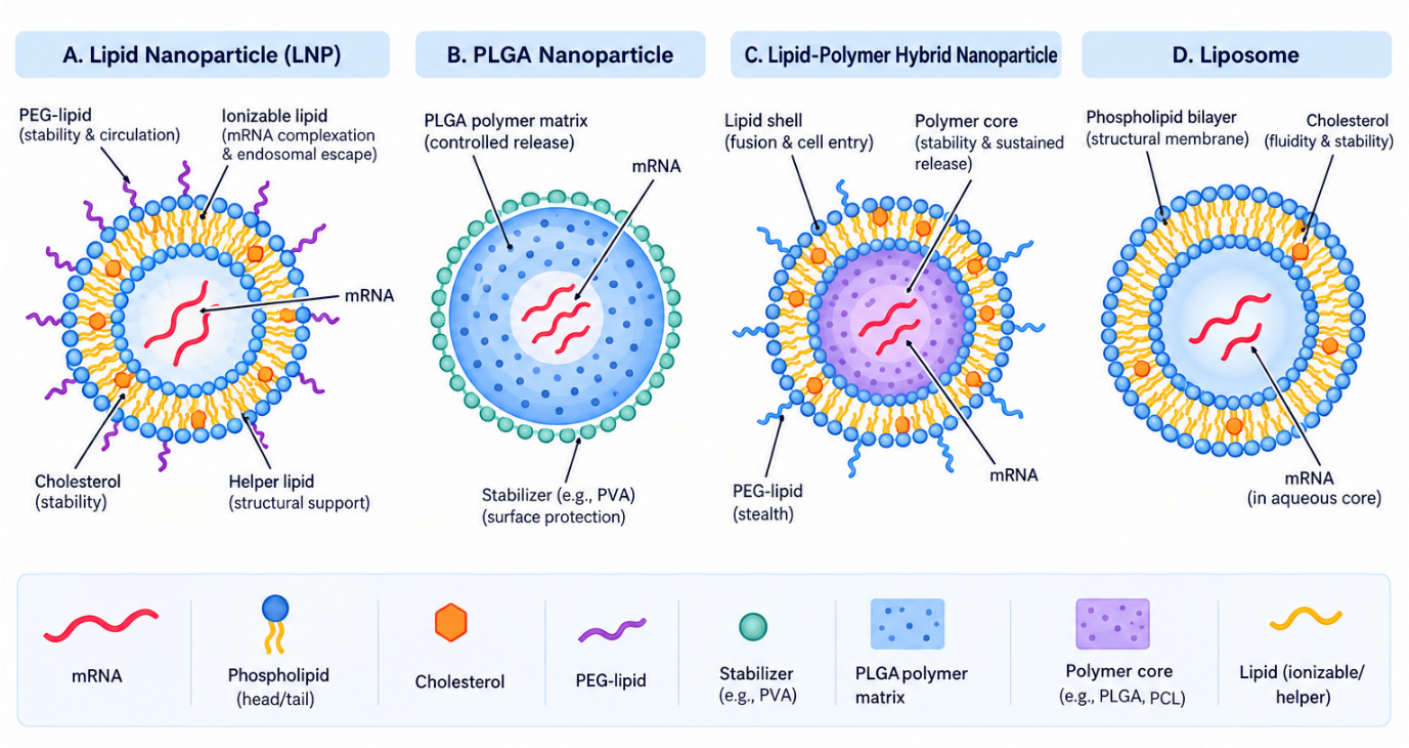


Figure 2: Architecture of mRNA Delivery Systems.

stop working a lesson used for vaccines. Special lipids such as DLin-MC3-DMA (MC3, Onpattro) SM-102 (mRNA-1273) and ALC-0315 (BNT162b2) have become standards each with ways of working in the body.

The amount of each LNP part affects how the system works how much mRNA is inside and how it works in the body. The best formulas for mRNA vaccines usually have a ratio of 50:10:38.5:1.5 (%) for the four parts, which makes more than 95% of the mRNA inside and has a nitrogen to phosphate ratio of 3-6 (Patel *et al.*, 2020). The size is kept between 80-150 nm, which helps the system get to the places in the body like the lymph nodes.

Newer versions of LNPs can send the message to organs by changing the lipids and adding parts that help it go to the right place, like the spleen (for making the immune system work better) the liver or the lungs (Sabnis *et al.*, 2018). Sending the message to the spleen is important for vaccines that fight cancer as the spleen is the place where the immune system starts.

Polymer-Based Systems

Liquids that have charges mainly Polyethylenimine (PEI) and Poly(Lactic-Co-Glycolic Acid) (PLGA) have been looked at as ways to carry mRNA (Kon *et al*., 2023). PEI makes groups with the mRNA and works well in the lab using a 'proton sponge' to help the message get out of the cells bubble (Kaczmarek *et al*., 2017). However PEIs high charge is linked to problems with the body at the right doses, which stops it from being used in people.

PLGA particles are safe break down over time and are well tolerated. They don't hold much mRNA and don't get out of the cells bubble very well which makes them not work as well (Patel *et al*., 2019). Mixing PLGA cores with parts that have charges helps with these problems by combining the structure of PLGA with the way lipids work.

Liposomes and Hybrid Systems

Older ways of using liposomes (lipoplexes) were the first to be studied for carrying mRNA but didn't work well because of how the body reacted, not taking the right cells and making people sick (Islam *et al*., 2018). Lipid-Polymer hybrid Nanoparticles (LPNs) combine the parts of PLGA with the way lipids work making them better at working in the lab and in the body than either part alone.

Targeted Delivery Approaches

Getting the message to the spot in the body through the EPR effect is not enough, for solid tumors so other ways are needed (Luo *et al*., 2023; Kimura *et al*., 2021). Changing the surface of LNPs with things that find the receptors in tumors (HER2, EGFR folate receptor, transferrin receptor) has shown better results in studies (Kaczmarek *et al*., 2017). Another way uses putting the mRNA-LNPs in the tumor with messages that help the immune system, which changes the environment in the tumor. This method avoids the problems of sending the message through the body. Is the way the mRNA-2752 clinical program work.

Challenges in Delivery

Despite improvements in LNP engineering getting the delivery right in solid tumors is still very hard. A major study showed that usually less than 2% of the mRNA-LNPs given get to the inside of the target cells with the rest stuck in endosomes or sent to lysosomes (Blanco *et al*., 2015). This big problem is the reason why mRNA cancer vaccines are not as strong as they could be at doses that are practical for patients.

Cellular Uptake Barriers: Getting into the cell by endocytosis works best when the LNP size is just right (80-200 nm) the surface charge is slightly negative or neutral (-10 to -5 mV zeta potential). There is no opsonization. Soon as the LNPs are put into the vein they quickly get covered in proteins, which changes their surface and sends them to the liver, spleen and other parts of the mononuclear phagocyte system. This reduces how much gets to the place (Gillmore *et al*., 2021).

Endosomal Escape: Getting out of the endosome is the problem for mRNA delivery. About 1-2% of the endosomal LNPs manage to break the membrane and release the mRNA into the cytoplasm (Zeng *et al*., 2022). When the ionizable lipid gets protonated at the pH of the endosome (5-6) it changes the shape of the lipid membrane making it unstable and creating small holes. Researchers are looking at ways to help the mRNA escape, such as using pH- lipids that help merge with the membrane adding cone-shaped lipids that make the membrane bend using special peptides that break the membrane and using light to help release the mRNA.

Target Specificity: When LNPs are given into the body they mostly end up in the liver because of the way sticks to PEGylated LNPs, which helps them get into liver cells (Ball *et al*., 2018). Changing the way the LNPs are made so they go to the tumor or to the lymph nodes instead is needed. This requires changing the surface the amount of PEG-lipid or adding targeting molecules. Each of these changes adds difficulty to the manufacturing process.

Toxicity Issues: The cationic lipids and much ionizable lipids at the wrong amounts can cause damage to the cell membranes affect the mitochondria and start inflammation (Guo *et al*., 2018; Nair *et al*., 1998). The PEG-lipids, even though they help keep the LNPs stable can cause the body to make antibodies against PEG. This is called 'Accelerated Blood Clearance' (ABC). It makes the LNPs less effective the second time they are given and it also causes problems with how well the body can handle repeated doses of the vaccine.

Stability Challenges

The weak nature of mRNA molecules is one of the problems in the field of pharmaceuticals. Therapeutic mRNA can break down in different ways, which makes it harder to keep and use in the body.

Enzymatic Degradation

RNases are everywhere in fluids like blood and in the spaces between cells. The mRNA without any protection only lasts than a minute in blood because of these enzymes (Sahin and Türeci, 2018). Putting the mRNA inside LNPs helps protect it from the enzymes reducing the breakdown by than 90%. However, some mRNA may still break down during the making or storing of the LNPs. This damage isn't easy to see with tests but it makes the mRNA not work as well when given to a patient (Table 2).

Table 2: Clinical Trials of mRNA Vaccines in Solid Tumors.

Vaccine (Sponsor)	Target/Antigen	Delivery	Cancer Type	Phase	Key Findings
mRNA-4157 (Moderna/MSD)	mRNA-4157/V940	LNP	Melanoma (adjuvant)	Phase 2b	44% RFS improvement; RECIST responses
BNT111 (BioNTech)	NY-ESO-1, MAGE-A3, TPTE, BAGE2	LNP	Advanced melanoma	Phase 2	Objective responses; durable T-cell responses
BNT122 (Gritstone/BioNTech)	Personalized neoantigen	LNP	Pancreatic/colorectal cancer	Phase 2	Neoantigen-specific T cells; disease stabilization
mRNA-2752 (Moderna)	OX40L, IL-23, IL-36γ	LNP	Solid tumors (IT)	Phase 1	TME reprogramming; tolerated dose
BNT131 (BioNTech)	IL-12, IL-15, GM-CSF	LNP	Solid tumors/lymphoma	Phase 1	Immune activation; ongoing
CV9202 (CureVac)	6 NSCLC antigens	Protamine	Non-small cell lung cancer	Phase 1/2	Disease stabilization in ~50%
mRNA-2416 (Moderna)	OX40L	LNP	Solid tumors/lymphoma	Phase 1	Ongoing; safety established

Chemical Degradation

The mRNA can break down because of the way the chemical parts are connected especially when it's in an alkaline environment or when it's hot. The extra 2′-OH groups on the sugar part of the RNA make it more likely to break down than DNA (Moderna press release, 2023). This means that the pH has to be carefully controlled (between 7.0 and 7.5) when making and storing the mRNA. Some changes to the nucleosides, which help lower the response can also help a little with chemical breakdown in certain parts of the mRNA.

Formulation Stability Strategies

Drying the mRNALNP solutions with things like trehalose, sucrose or mannitol at 5-15% (w/v) is the best way to make them stable at room temperature or 4°C (Palmer *et al*., 2022; Awasthi *et al*., 2021). This could help avoid the need for cold storage. Other methods like spray-drying and vacuum-foam drying are being studied as options that work with continuous production. Lipid oxidation is a problem that is not talked about much but is important in life. This is handled by using nitrogen during the making and adding antioxidants like vitamin.

Cold Chain Dependency

The BNT162b2 vaccine needs to stay at -70°C and the mRNA-1273 needs to stay at -20°C. These requirements are hard to manage and cost a lot of money which makes it hard to get the vaccines to people around the world (Iavarone *et al*., 2017). Making mRNA-LNP formulations that can stay stable at 2-8°C for least six months is a big goal, for research. Recently it was shown that dried mRNA-LNPs can stay stable at 25°C for 30 days, which suggests that it is possible to make vaccines that work at room temperature in the future.

Pharmacokinetics and Biodistribution

The Pharmacokinetic (PK) behavior of mRNA-LNP systems is very different from that of molecules and biologics. The active part is not the mRNA itself but the protein antigen that is made from it. This creates a delay and an amplification step in the way dose and response are related (Shi and Lammers, 2019; Beck *et al*., 2021).

Distribution Patterns

After a IM) injection the usual way cancer vaccines are given-mRNA-LNPs mainly go to the muscle at the injection site the draining lymph nodes and the spleen (Lorenzi *et al*., 2022). A small but important part of the LNPs moves through the lymph system to the lymph nodes, where dendritic cells present the antigen (Kreiter *et al*., 2015). Studies in rodents that use luciferase-encoding mRNA show that than 95% of the antigen is made at the injection site with only 1 to 3% in the draining lymph nodes and less than 0.5% in the spleen. This shows how important local antigen presentation is for activating T cells.

Clearance

mRNA is removed from the body through parallel routes. Inside the cell it is broken down by nucleases when the endosome ruptures. In the cytoplasm RNA decay machinery-such as decapping enzymes, exonucleases and XRN1-degrades it. The immune system also destroys cells that have taken up the mRNA (Gubin *et al*., 2014; Ott *et al*., 2017). Because of these routes the antigen that is made is short-lived. It usually reaches its peak between 6 and 24 hr after injection. Falls back to background levels within 3 to 7 days for regular mRNA (Van Hoecke *et al*., 2020).

Table 3: Stability Challenges and Formulation Mitigation Strategies for mRNA-LNP Systems.

Stability Challenge	Mechanism	Formulation Strategy	Outcome/Status
mRNA enzymatic degradation (RNase)	Ubiquitous RNases on skin/serum	Encapsulation in LNPs; modified nucleosides; HEPA-filtered GMP	Prevents >90% RNase-mediated degradation
mRNA hydrolysis (chemical)	Phosphodiester bond cleavage at alkaline pH	Lyophilization; pH-controlled formulation (pH 7-7.5); cryoprotectants (trehalose, sucrose)	Extended shelf life at -20°C to 4°C
mRNA denaturation	Thermal exposure, freeze-thaw cycles	Stable LNP shell; lyophilization; mRNA secondary structure optimization	Critical for distribution logistics
LNP structural instability	Fusion/aggregation during storage	PEGylation; optimal lipid ratio; cryoprotectant addition	Maintains uniform particle size (PDI <0.2)
Immunostimulation by dsRNA	*In vitro* transcription by-products	HPLC purification; column chromatography; modified nucleosides	Reduces innate immune activation significantly
Cold chain dependency	Lipid phase transition above critical temperature	Thermostable LNP formulations; lyophilized formats; room-temp stable excipients	Active research area; key barrier for global access

Dose Considerations

mRNA vaccine pharmacokinetics is not linear unlike small-molecule PK because cells can only take up an amount of mRNA the immune response depends on the dose and the lipids can become toxic at higher doses (Gallie, 1991). In trials for cancer neoantigen vaccines, doses of 0.1 to 1.0 mg per antigen were used. The decision to increase the dose was based on how strong the T-cell response was, how well the patient tolerated the vaccine and the patterns of cytokine release not on PK data.

Preclinical and Clinical Progress

Preclinical Findings

Studies in animals have set basic rules for mRNA cancer vaccines. Personalized neoantigen mRNA vaccines that carry proven mutations reliably trigger CD4+ and CD8+ T-cell responses that target the neoantigen in mouse tumors such as MC38 (colorectal) CT26 and B16F10 (melanoma) (Holtkamp *et al*., 2006). These vaccines can shrink tumors. Improve survival in both preventive and treatment situations especially when used together with anti-PD-1 checkpoint blockers (Table 3) (Mauger *et al*., 2019).

Mechanistic studies show that: (i) the physical and chemical traits of LNPs strongly affect how well dendritic cells are targeted and how long the LNPs stay in the lymph nodes; (ii) delivering LNPs to the spleen via injection of slightly negatively charged LNPs produces stronger systemic CTL responses than intramuscular injection; and (iii) injecting LNPs directly into the tumor with immunostimulatory cytokines such as IL-12 and GM-CSF reshapes the Tumor Microenvironment (TME) and works well with systemic checkpoint blockade (Table 4) (Anderson *et al*., 2010).

Clinical Trials in Solid Tumors

The leading mRNA cancer vaccine in development is mRNA-4157/V940 from Moderna and Merck. It is a neoantigen vaccine that can carry up to 34 different neoantigens for each patient and it is packaged in LNPs (Anderson *et al*., 2010). In a Phase 2b study called KEYNOTE-942 mRNA-4157/V940 given after surgery with pembrolizumab lowered the chance of tumor recurrence or death by 44% compared with pembrolizumab alone in patients who had high-risk melanoma that had been removed. This was the randomised controlled study that showed a clear benefit for an mRNA cancer vaccine (Svitkin *et al*., 2017). Because of this breakthrough the programme has grown to include trials for Non-Small-Cell Lung Cancer (NSCLC) and renal cell carcinoma.

BNT111 from BioNTech carries four melanoma antigens-NY-ESO-1, MAGE-A3, TPTE and BAGE2 delivered in LNPs. In a Phase 2 trial it produced responses, including one complete response in melanoma patients who had previously been treated with anti-PD-1 (Durymanov and Reineke, 2018). BNT122 aims at neoantigens for pancreatic and colorectal cancer. It has shown that patients with Pancreatic Ductal Adenocarcinoma (PDAC) a cancer that is usually hard to treat with vaccines develop T cells that target the neoantigens (Gilleron *et al*., 2013; Paunovska *et al*., 2022).

Vaccines given directly into tumors, such as mRNA-2752 from Moderna which carries OX40L, IL-23 and IL-36γ and BNT131

Table 4: Clinical Development Pipeline of mRNA Cancer Vaccines for Solid Tumors (2017-2025).

Program	Trial ID	Tumor setting	Start	End	Reported stage	Clinical timeline
Autogene cevumeran (RO7198457)	NCT03289962	Advanced solid tumors	2017	2025	Phase 1	2017-2025
mRNA-4157 / V940	NCT03313778	Solid tumors / melanoma	2017	2025	Phase 1 → Phase 2/2b; Phase 3 programs	2017-2025
mRNA-5671 / V941	NCT03948763	KRAS-mutant solid tumors	2019	2022	Phase 1	2019-2022
BNT111	NCT04526899	Advanced melanoma	2021	2025	Phase 2	2021-2025
BNT113	NCT04534205	HPV16+ head and neck cancer	2021	2025	Phase 2/3	2021-2025

from BioNTech which carries IL-12, IL-15 and GM-CSF have shown that the tumor microenvironment can be changed and that the immune system becomes active. These results came from Phase 1 trials in different solid cancers and the safety was acceptable (Tenchov *et al*., 2021; Samaridou *et al*., 2020).

Safety and Efficacy Trends

In all studies mRNA cancer vaccines have shown a steady and safe safety pattern. The common side effects are reactions at the injection site redness and tenderness plus general symptoms such as tiredness, fever, muscle aches and short-term drops in blood cell counts. These are in line with how the vaccine works and usually go away on their own (Reichmuth *et al*., 2016). Serious problems that can be directly linked to the mRNA vaccines are very uncommon. No cases of cancer caused by the vaccine no integration, into the germline and no long-term autoimmune disease have been seen. This matches the fact that the vaccine causes a temporary expression and does not join the DNA. Representative clinical programs; timeline indicates reported clinical-development periods and is not an exhaustive trial census.

Interpretation of the timeline

Key interpretation for the review

- Clinical development expanded from early dose/safety studies toward randomized and pivotal programs during 2017-2025.
- Programs differ in antigen strategy and tumor setting, including individualized neoantigens, shared tumor antigens and KRAS-directed approaches.
- The timeline describes development stage, not comparative efficacy, regulatory approval or clinical benefit.

Source note: Representative trial records and published clinical reviews. Trial status and development stage may change as studies are updated. The figure should be cited alongside the primary trial records used in the manuscript.

Combination Strategies

With Immune Checkpoint Inhibitors

Combining mRNA cancer vaccines with PD-1/PD-L1 or CTLA-4 checkpoint blockers is the proven and logical strategy. Checkpoint inhibitors stop T cells from getting tired. Help them kill cancer cells again while the mRNA cancer vaccine makes more T cells that recognize the cancer. The KEYNOTE-942 data showing that mRNA-4157/V940 with pembrolizumab works better than pembrolizumab gives the first randomized proof of this synergy. The order and timing of the combination can be very important. Studies in animals suggest that giving the mRNA cancer vaccine first then the checkpoint blocker, can grow cancer-recognizing T cells before re-activating tired T cells maybe giving better results than giving both at the same time (Tros de *et al*., 2010).

With Chemotherapy

Some chemotherapy drugs like cyclophosphamide, gemcitabine and oxaliplatin can kill cancer cells in a way that alerts the system. They release tumor parts danger signals such as calreticulin and HMGB1 and inflammatory chemicals that help the mRNA cancer vaccine work better (Kowalski *et al*., 2019). Also giving doses of chemo over time can reduce Tregs and MDSCs which normally suppress the immune system in the tumor area. Clinical studies that combine mRNA cancer vaccines with chemotherapy are still few. This area is being actively explored, especially for tumors that are normally ”cold” to the immune system.

With Radiotherapy

Radiation makes the tumor area inflamed increases MHC class I on cancer cells. Releases antigens that let the immune system learn to attack the tumor. The abscopal effect-when radiation causes non-irradiated tumors to shrink through systemic immune activation is much stronger when a cancer vaccine is given at the same time. MRNA cancer vaccines wrapped in Lipid Nanoparticles (LNPs) that produce immune-boosting cytokines when paired with radiation can turn tumors that hide from the immune system into ones that react to it making checkpoint blockers work better.

Manufacturing and Formulation Considerations

Making personalized neoantigen mRNA cancer vaccines in GMP labs involves four steps: (1) reading the patient's DNA and RNA (WES/RNA-seq); (2) picking the neoantigens and designing the vaccine; (3) producing the mRNA in GMP conditions using *in vitro* transcription; and (4) putting the mRNA into lipid nanoparticles. Key quality measures for the LNP product are: mRNA inside the particles more than 90%, particle size 80-150 nanometers with a smooth distribution (PDI below 0.2) mRNA that is mostly full length (above 90% by gel test) low leftover solvents, endotoxin under 5 EU per kg and no microbes. Scale-Up Challenges: Microfluidic mixing is the way It mixes amounts of lipid-ethanol and mRNA-water quickly to form uniform particles. The machines that do this can only handle a small amount at a time and because each patient needs a different vaccine, we face special GMP challenges that normal drugs do not have. New high-throughput automated machines that can do same-day GMP mRNA production and LNP formulation are being worked on. Regulatory Aspects: mRNA cancer vaccines sit in a regulatory area. In the US they are called products (21 CFR Part 600). In Europe they are Therapy Medicinal Products (ATMPs). In some places they are gene therapy products. Regulators want to see that the mRNA sequence is correct that each LNP batch is the same that we control the process, for products and that we have plans to watch for safety after approval. The FDA's Accelerated Approval and Breakthrough Therapy programs have helped speed up the development of mRNA-4157(Dowdy, 2017).

Comparative Analysis: mRNA vs. Other Vaccine Platforms

A careful comparison of mRNA vaccines with vaccine types helps to understand where mRNA vaccines fit in cancer immunotherapy (Table 5). MRNA vs. DNA Vaccines: DNA vaccines have some strengths. They are more stable at temperatures. They do not need storage. They also can keep producing antigens for a time. DNA vaccines must get into the cell nucleus to work. This is hard in cells that are not dividing. There is also a risk that DNA could mix into the cell's own genes. This risk is very low but still possible. The bigger problem is that DNA vaccines have not worked well in trials. Their real-world performance has been much weaker than what was seen in lab studies. This is because it is hard for DNA to get into the nucleus and start making protein. MRNA vaccines do not need to go into the nucleus. They work directly in the cytoplasm. They show immune responses in people. They have already been proven safe and effective in use. That makes mRNA vaccines better suited for cancer vaccines even though they need cold storage.

MRNA vs. Viral Vectors: Viral vectors like adenovirus and poxvirus can deliver material very well. They also trigger immune responses on their own. They come with serious problems. Many people already have immunity to these viruses. This is especially true for adenovirus 5 and chimpanzee adenovirus. This can make the vaccine less effective. These vectors can also cause inflammation. They are hard to make. They require safety levels like BSL2. They are not easy to change for each person. MRNA vaccines are different. They are simple to change for each patient. They can be made in labs following good manufacturing practices. They do not rely on a virus so there is no issue with pre-existing immunity. These features make mRNA vaccines much better for cancer vaccines.

MRNA vs. Peptide Vaccines: Peptide vaccines are simple and safe. They target responses in a precise way. They need HLA testing first. They only work with types of HLA. They are limited to an HLA types. Also, short peptides usually only activate CD8+ T cells. They do not activate CD4+ T helper cells. Long peptide vaccines help some of these problems. They still depend on how well dendritic cells present the peptides. MRNA vaccines naturally work in a way that activates both MHC class I and MHC class II pathways. This means they can activate both killer T cells and helper T cells. They do not need HLA testing. This makes them stronger and more flexible, in cancer treatment (Crooke *et al*., 2018).

Current Limitations

Delivery Inefficiency: Even though LNP technology has improved, the problem of endosomal entrapment remains. This means that about 1 to 2% of the mRNA reaches the cytoplasm after a dose is given. This low delivery rate requires doses of LNPs, which brings up issues related to safety and increases production costs. The active area in LNP research right now is the development of next-generation ionizable lipids. These are being designed to improve activity meaning they help break out of endosomes more effectively. Researchers use models and high-throughput screening to guide this work (Schlake *et al*., 2012). Tumor Heterogeneity: Solid tumors are not uniform. They show differences in antigen expression both in space and over time. When vaccines target neoantigens the immune system may eliminate some cancer cells but leave behind those that have lost the targeted antigens.

FUTURE PERSPECTIVES

Personalized mRNA Vaccines and AI Integration

Artificial intelligence is expected to transform steps in the mRNA cancer vaccine pipeline. Deep learning models are already improving how we select neoantigens. These models predict how tightly peptides bind to MHC molecules, how T-cell receptors interact with antigens and how likely an antigen is to provoke a response. This helps reduce positives and allows for more accurate vaccine design (Liu, 2019). AI is also being used to optimize mRNA sequencesfinding the codon choices, optimal untranslated regions and secondary structures that boost protein

Table 5: Comparative Analysis: mRNA Vaccines vs. Competing Vaccine Platforms.

Parameter	mRNA Vaccine	DNA Vaccine	Viral Vector	Peptide Vaccine
Integration risk	None	Possible (rare)	Possible	None
Nuclear entry required	No	Yes	No	No
Expression onset	Rapid (hrs)	Delayed (days)	Variable	Rapid (hrs)
Expression duration	Transient (days-weeks)	Long (weeks-months)	Long (months-years)	Transient (days)
Immunogenicity	Low-moderate (modified)	Low	High (pre-existing immunity)	Low (protein-based)
Manufacturing	GMP-scalable	Moderate complexity	Complex/BSL2-3	Complex
Cold chain	-old chainL2-3	2-8°C	–8°C chainL2-3exit	2-8°C
Personalization	Highly feasible (rapid)	Possible	Difficult	Possible
Clinical maturity	Approved (COVID-19)	Multiple approved	Multiple approved	Early stage
Cost	Moderate-high	Moderate	High	High

production. Some commercial platforms now include these AI-driven optimizations. Looking forward integrating biopsies especially circulating tumor DNA data could allow real-time updates to vaccines as the tumor changes creating a truly adaptive form of personalized immunotherapy.

Advanced Nanocarrier Development

New LNP platforms are being developed to overcome limitations. One focus is on lipids that naturally break out of endosomes more efficiently than today's versions. Another approach uses SORT-LNPsmodified LNPs that target organs like the spleen or lymph nodes leading to better T-cell activation after systemic injection. There are also stimuli- LNPs that release their payload in response to conditions found in the tumor microenvironment, such as low pH, high redox activity or specific enzymes. Addition multi-cargo LNPs are being explored to deliver both antigen mRNA and immunostimulatory adjuvant mRNA in precise ratios for enhanced immune effects. Beyond lipid-based systems non-lipid delivery platforms are emerging. Polymersomes, virus- particles and exosomes derived from cells offer different advantages. E.g. they may avoid issues like -PEG immune reactions seen with some lipid nanoparticles. These alternatives could address weaknesses of current lipid systems and expand the range of possible delivery strategies (Table 6).

Clinical Translation Improvements

For the decade key priorities in clinical translation include: (i) cutting down the time it takes to make personalized vaccines from 4 to 6 weeks to less than 2 weeks-so they can be used in minimal residual disease or adjuvant settings; (ii) developing thermostable mRNA-LNP formulations so ultra-cold storage is no longer needed; (iii) identifying predictive biomarkers like HLA diversity, tumor mutational burden, neoantigen clonality and pre-existing T-cell repertoire to better select patients who will benefit; (iv) using adaptive trial designs that allow faster detection of combination therapy signals; and (v) conducting health economics studies to support fair reimbursement models, for personalized biologic products (Beissert *et al*., 2020).

CONCLUSION

Engineering mRNA vaccines for tumor immunotherapy is one of the active and interesting areas in pharmaceutical science. Over the ten years there has been great progress. Early breakthroughs in nucleoside modification made mRNA silent to the system and improved its translation. Then scientists created lipid nanoparticle delivery systems. The first clinical proof showed that mRNA vaccines can improve recurrence-free survival in controlled trials. This review has carefully examined the engineering concepts behind mRNA vaccine development focusing on delivery challenges that remain obstacles to real-world use. Current lipid nanoparticle systems escape the endosome 1 to 2% of the time. Inside the body mRNA vaccines face a tumor microenvironment that blocks their actions. The mRNA molecule itself is fragile. Degrades easily. There are also cold-chain requirements for storage and transport and high costs linked to manufacturing. These factors together form barriers that slow adoption. Overcoming them will need progress across formulation chemistry, nanotechnology, computational biology and improvements in how mRNA vaccine made at scale.

Importantly new clinical results especially the Phase 2b KEYNOTE-942 trial showing a 44% improvement in recurrence-free survival when using mRNA-4157/V940 in melanoma confirm that mRNA cancer vaccines can lead to patient benefits. Extending this success to tumors that are currently hard to treat such as pancreatic cancer, glioblastoma and microsatellite-stable

Table 6: List of Abbreviations.

Abbreviations	Full Form	Context/Note
LNP	Lipid Nanoparticle	Primary mRNA delivery vehicle; ionizable lipid-based
TME	Tumor Microenvironment	Immunosuppressive milieu surrounding solid tumors
MHC	Major Histocompatibility Complex	Antigen presentation molecule (class I and II)
CTL	Cytotoxic T Lymphocyte	CD8+ effector T cell mediating tumor cell killing
APC	Antigen-Presenting Cell	Dendritic cells, macrophages; bridge innate-adaptive immunity
DC	Dendritic Cell	Professional APC; mRNA vaccine primary target cell
PLGA	Poly (lactic-co-glycolic acid)	Biodegradable polymer for nanoparticle delivery
PEG	Polyethylene Glycol	Steric stabilizer; reduces opsonization of nanoparticles
IVT	*In vitro* Transcription	Enzymatic method for mRNA synthesis from DNA template
ψ (Ψ)	Pseudouridine	Modified nucleoside; reduces innate immune activation
m1Ψ	N1-methyl pseudouridine	Key modification in BNT162b2 and mRNA-1273 vaccines
dsRNA	Double-stranded RNA	IVT by-product; activates innate immune sensors (TLR3, PKR)
EPR	Enhanced Permeability and Retention	Passive tumor accumulation of nanoparticles via leaky vasculature
PD-1/PD-L1	Programmed Death-1/Ligand-1	Immune checkpoint pathway; combined with mRNA vaccines
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4	Immune checkpoint on T cells; target for combination therapy
NSCLC	Non-Small Cell Lung Cancer	Most common lung cancer type; target for mRNA vaccines
PDAC	Pancreatic Ductal Adenocarcinoma	Highly aggressive solid tumor; subject of mRNA neoantigen trials
IT	Intratumoral	Route of administration; direct injection into tumor mass
GMP	Good Manufacturing Practice	Regulatory standard for pharmaceutical production
RFS	Recurrence-Free Survival	Primary endpoint in mRNA-4157/V940 Phase 2b trial

colorectal cancers will require stronger delivery methods, better ways to change the tumor environment into a more supportive state for immune attack and smarter combination therapies guided by biomarkers that help identify which patients are most likely to respond. The merging of mRNA technology with intelligence to design neoantigens, next-generation lipid nanoparticle engineering and well-planned immunotherapy combinations suggests that mRNA vaccines are becoming a tool in precision oncology. From the perspective of pharmaceutical science, the field is shifting from proving that mRNA vaccines work to making them better. The problems related to formulation, delivery and stability are now clear and within reach of solution. It seems likely that over the decade mRNA cancer vaccines will become a standard part of adjuvant treatment plans, for many types of solid tumors.

ABBREVIATIONS

LNP: Lipid Nanoparticle; **TME:** Tumor Microenvironment; **MHC:** Major Histocompatibility Complex; **CTL:** Cytotoxic T Lymphocyte; **APC:** Antigen-Presenting Cell; **DC:** Dendritic Cell; **PLGA:** Poly(lactic-co-glycolic acid); **PEG:** Polyethylene Glycol; **IVT:** *In vitro* Transcription; **ψ (Ψ):** Pseudouridine; **m1Ψ:** N1-methylpseudouridine; **dsRNA:** Double-Stranded RNA; **EPR:** Enhanced Permeability and Retention; **PD-1/PD-L1:** Programmed Death-1/Programmed Death-Ligand 1; **CTLA-4:** Cytotoxic T-Lymphocyte-Associated Protein 4; **NSCLC:** Non-Small Cell Lung Cancer; **PDAC:** Pancreatic Ductal Adenocarcinoma; **IT:** Intratumoral; **GMP:** Good Manufacturing Practice; **RFS:** Recurrence-Free Survival.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research received no external funding.

REFERENCES

Akinc, A., Maier, M. A., Manoharan, M., *et al*. (2019). The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nature Nanotechnology*, *14*(12), 1084-1087. https://doi.org/10.1038/s41565-019-0591-y

Anderson, B. R., Muramatsu, H., Nallagatla, S. R., *et al*. (2010). Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. *Nucleic Acids Research*, *38*(17), 5884-5892. https://doi.org/10.1093/nar/gkq347

Awasthi, S., Balliet, J. W., Flynn, J. A., *et al*. (2021). Protection against acquisition of genital HSV-2 with an mRNA-1608/AS01B vaccine. *Science Immunology*, *6*(59), eabd4047. https://doi.org/10.1126/sciimmunol.abd4047

Awasthi, S., Vekariya, R. H., & Mittal, M. (2022). mRNA vaccines for cancer treatment. *Vaccines*, *10*(11), 1916. https://doi.org/10.3390/vaccines10111916

Ball, R. L., Hajj, K. A., Vizelman, J., *et al*. (2018). Lipid nanoparticle formulation for *in vivo* mRNA delivery. *Nano Letters*, *18*(6), 3814-3822. https://doi.org/10.1021/acs.nanolett.8b01101

Beck, J. D., Reidenbach, D., Salomon, N., *et al*. (2021). mRNA therapeutics in cancer immunotherapy. *Molecular Cancer*, *20*(1), 69. https://doi.org/10.1186/s12943-021-01348-0

Beissert, T., Perkovic, M., Vogel, A., *et al*. (2020). A trans-amplifying RNA vaccine strategy for induction of potent protective immunity. *Molecular Therapy*, *28*(1), 119-128. https://doi.org/10.1016/j.ymthe.2019.09.009

Binnewies, M., Roberts, E. W., Kersten, K., *et al*. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nature Medicine*, *24*(5), 541-550. https://doi.org/10.1038/s41591-018-0014-x

Blanco, E., Shen, H., & Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nature Biotechnology*, *33*(9), 941-951. https://doi.org/10.1038/nbt.3330

Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity*, *39*(1), 1-10. https://doi.org/10.1016/j.immuni.2013.07.012

Crooke, S. T., Witztum, J. L., Bennett, C. F., & Baker, B. F. (2018). RNA-targeted therapeutics. *Cell Metabolism*, *27*(4), 714-739. https://doi.org/10.1016/j.cmet.2018.03.004

Dowdy, S. F. (2017). Overcoming cellular barriers for RNA therapeutics. *Nature Biotechnology*, *35*(3), 222-229. https://doi.org/10.1038/nbt.3802

Durymanov, M., & Reineke, J. (2018). Non-viral delivery of nucleic acids: Insight into mechanisms of overcoming intracellular barriers. *Frontiers in Pharmacology*, *9*, Article 971. https://doi.org/10.3389/fphar.2018.00971

Gallie, D. R. (1991). The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes & Development*, *5*(11), 2108-2116. https://doi.org/10.1101/gad.5.11.2108

Gilleron, J., Querbes, W., Zeigerer, A., *et al*. (2013). Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. *Nature Biotechnology*, *31*(7), 638-646. https://doi.org/10.1038/nbt.2612

Gillmore, J. D., Gane, E., Taubel, J., *et al*. (2021). CRISPR-Cas9 *in vivo* gene editing for transthyretin amyloidosis. *New England Journal of Medicine*, *385*(6), 493-502. https://doi.org/10.1056/NEJMoa2107454

Gubin, M. M., Zhang, X., Schuster, H., *et al*. (2014). Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*, *515*(7528), 577-581. https://doi.org/10.1038/nature13988

Guo, P., Liu, D., Subramanyam, K., *et al*. (2018). Nanoparticle elasticity directs tumor uptake and retention mediating drug efficacy. *Nature Communications*, *9*(1), 130. https://doi.org/10.1038/s41467-017-02588-9

Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discovery*, *12*(1), 31-46. https://doi.org/10.1158/2159-8290.CD-21-1059

Hassett, K. J., Benenato, K. E., Jacquinet, E., *et al*. (2019). Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines. *Molecular Therapy-Nucleic Acids*, *15*, 1-11. https://doi.org/10.1016/j.omtn.2019.01.013

Hegde, P. S., & Chen, D. S. (2020). Top 10 challenges in cancer immunotherapy. *Immunity*, *52*(1), 17-35. https://doi.org/10.1016/j.immuni.2019.12.011

Holtkamp, S., Kreiter, S., Selmi, A., *et al*. (2006). Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. *Blood*, *108*(13), 4009-4017. https://doi.org/10.1182/blood-2006-04-015024

Hou, X., Zaks, T., Langer, R., & Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. *Nature Reviews Materials*, *6*(12), 1078-1094. https://doi.org/10.1038/s41578-021-00358-0

Iavarone, C., O'Hagan, D. T., Yu, D., *et al*. (2017). Mechanism of action of mRNA-based vaccines. *Expert Review of Vaccines*, *16*(9), 871-881. https://doi.org/10.1080/14760584.2017.1355245

Islam, M. A., Xu, Y., Tao, W., *et al*. (2018). Restoration of tumour-growth suppression *in vivo* via systemic nanoparticle-mediated delivery of PTEN mRNA. *Nature Biomedical Engineering*, *2*(11), 850-864. https://doi.org/10.1038/s41551-018-0284-0

Jayaraman, M., Ansell, S. M., Mui, B. L., *et al*. (2012). Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing *in vivo*. *Angewandte Chemie International Edition*, *51*(34), 8529-8533. https://doi.org/10.1002/anie.201203263

Kaczmarek, J. C., Kowalski, P. S., & Anderson, D. G. (2017). Advances in the delivery of RNA therapeutics. *Genome Medicine*, *9*(1), 60. https://doi.org/10.1186/s13073-017-0450-0

Karikó, K., Buckstein, M., Ni, H., & Weissman, D. (2005). Suppression of RNA recognition by Toll-like receptors: The impact of nucleoside modification. *Immunity*, *23*(2), 165-175. https://doi.org/10.1016/j.immuni.2005.06.008

Karikó, K., Muramatsu, H., Welsh, F. A., *et al*. (2008). Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity. *Molecular Therapy*, *16*(11), 1833-1840. https://doi.org/10.1038/mt.2008.200

Kimura, S., Khalil, I. A., Elewa, Y. H. A., & Harashima, H. (2021). Novel lipid combination for delivery of plasmid DNA to immune cells in the spleen. *Journal of Controlled Release*, *330*, 753-764. https://doi.org/10.1016/j.jconrel.2021.01.002

Kon, E., Ad-El, N., Hazan-Halevy, I., *et al*. (2023). Targeting cancer with mRNA-lipid nanoparticles. *Nature Nanotechnology*, *18*(9), 1063-1077. https://doi.org/10.1038/s41565-023-01464-6

Kowalski, P. S., Rudra, A., Miao, L., & Anderson, D. G. (2019). Delivering the messenger: Advances in technologies for therapeutic mRNA delivery. *Molecular Therapy*, *27*(4), 710-728. https://doi.org/10.1016/j.ymthe.2019.02.012

Kreiter, S., Vormehr, M., van de Roemer, N., *et al*. (2015). Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature*, *520*(7549), 692-696. https://doi.org/10.1038/nature14426

Kulkarni, J. A., Witzigmann, D., Thomson, S. B., *et al*. (2021). The current landscape of nucleic acid therapeutics. *Nature Nanotechnology*, *16*(6), 630-643. https://doi.org/10.1038/s41565-021-00898-0

Liu, C., Shi, Q., Huang, X., *et al*. (2023). mRNA-based cancer therapeutics. *Nature Reviews Cancer*, *23*(8), 526-543. https://doi.org/10.1038/s41568-023-00586-2

Liu, M. A. (2019). A comparison of plasmid DNA and mRNA as vaccine technologies. *Vaccines*, *7*(2), 37. https://doi.org/10.3390/vaccines7020037

Lorenzi, J. C. C., Tran, T. M. H., Dossa, R. G., *et al*. (2022). Neoantigen mRNA cancer vaccine. *Science Translational Medicine*, *14*(644), eabl4721. https://doi.org/10.1126/scitranslmed.abl4721

Luo, X., Peng, X., Hou, J., *et al*. (2023). Fluorinated lipid nanoparticles for enhancing mRNA delivery efficiency. *Advanced Functional Materials*, *33*(4), 2213032. https://doi.org/10.1002/adfm.202213032

Mahase, E. (2023). mRNA cancer vaccine cuts risk of melanoma recurrence in trial. *BMJ*, *380*, p308. https://doi.org/10.1136/bmj.p308

Maier, M. A., Jayaraman, M., Matsuda, S., *et al*. (2013). Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Molecular Therapy*, *21*(8), 1570-1578. https://doi.org/10.1038/mt.2013.103

Mauger, D. M., Cabral, B. J., Presnyak, V., *et al*. (2019). mRNA structure regulates protein expression through changes in functional half-life. *Proceedings of the National Academy of Sciences*, *116*(48), 24075-24083. https://doi.org/10.1073/pnas.1908052116

Miao, L., Zhang, Y., & Huang, L. (2021). mRNA vaccine for cancer immunotherapy. *Molecular Cancer*, *20*(1), 41. https://doi.org/10.1186/s12943-021-01335-5

Moderna, Inc. (2023). *Moderna and Merck announce positive updated results from phase 2b study of investigational personalized cancer vaccine (mRNA-4157/V940) in combination with Keytruda® (pembrolizumab) in patients with high-risk melanoma* [Press release]. https://investors.modernatx.com/news-releases/news-release-details/moderna-and-merck-announce-positive-updated-results-phase-2b

Nair, S. K., Boczkowski, D., Morse, M., *et al*. (1998). Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes *in vitro* using human dendritic cells transfected with RNA. *Nature Biotechnology*, *16*(4), 364-369. https://doi.org/10.1038/nbt0498-364

Ott, P. A., Hu, Z., Keskin, D. B., *et al*. (2017). An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*, *547*(7662), 217-221. https://doi.org/10.1038/nature22991

Palmer, C. D., Rappaport, A. R., Davis, M. J., *et al*. (2022). Individualized, heterologous chimpanzee adenovirus and self-amplifying mRNA neoantigen vaccine for advanced metastatic solid tumors. *Nature Medicine*, *28*(8), 1619-1629. https://doi.org/10.1038/s41591-022-01937-6

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines-A new era in vaccinology. *Nature Reviews Drug Discovery*, *17*(4), 261-279. https://doi.org/10.1038/nrd.2017.243

Patel, A. K., Kaczmarek, J. C., Bose, S., *et al*. (2019). Inhaled nanoformulated mRNA polyplexes for protein production in lung epithelium. *Advanced Materials*, *31*(8), e1805116. https://doi.org/10.1002/adma.201805116

Patel, S., Ashwanikumar, N., Robinson, E., *et al*. (2020). Naturally-occurring cholesterol analogues in lipid nanoparticles induce polymorphic shape and enhance intracellular delivery of mRNA. *Nature Communications*, *11*(1), 983. https://doi.org/10.1038/s41467-020-14527-2

Paunovska, K., Loughrey, D., & Dahlman, J. E. (2022). Drug delivery systems for RNA therapeutics. *Nature Reviews Genetics*, *23*(5), 265-280. https://doi.org/10.1038/s41576-021-00439-4

Pollard, C., Rejman, J., De Haes, W., *et al*. (2013). Type I IFN counteracts the induction of antigen-specific immune responses by lipid-based delivery of mRNA vaccines. *Molecular Therapy*, *21*(1), 251-259. https://doi.org/10.1038/mt.2012.202

Reichmuth, A. M., Oberli, M. A., Jaklenec, A., *et al*. (2016). mRNA vaccine delivery using lipid nanoparticles. *Therapeutic Delivery*, *7*(5), 319-334. https://doi.org/10.4155/tde-2016-0006

Rojas, L. A., Sethna, Z., Soares, K. C., *et al*. (2023). Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature*, *618*(7963), 144-150. https://doi.org/10.1038/s41586-023-06063-y

Sabado, R. L., & Bhardwaj, N. (2015). Dendritic cell immunotherapy. *Annals of the New York Academy of Sciences*, *1352*(1), 1-12. https://doi.org/10.1111/nyas.12811

Sabnis, S., Kumarasinghe, E. S., Salerno, T., *et al*. (2018). A novel amino lipid series for mRNA delivery. *Molecular Therapy*, *26*(6), 1509-1519. https://doi.org/10.1016/j.ymthe.2018.03.010

Sahin, U., & Türeci, Ö. (2018). Personalized vaccines for cancer immunotherapy. *Science*, *359*(6382), 1355-1360. https://doi.org/10.1126/science.aar7112

Sahin, U., Derhovanessian, E., Miller, M., *et al*. (2017). Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*, *547*(7662), 222-226. https://doi.org/10.1038/nature23003

Samaridou, E., Heyes, J., & Lutwyche, P. (2020). Lipid nanoparticles for nucleic acid delivery. *Advanced Drug Delivery Reviews*, *154-155*, 37-63. https://doi.org/10.1016/j.addr.2020.06.002

Schlake, T., Thess, A., Fotin-Mleczek, M., & Kallen, K. J. (2012). Developing mRNA-vaccine technologies. *RNA Biology*, *9*(11), 1319-1330. https://doi.org/10.4161/rna.22269

Semple, S. C., Akinc, A., Chen, J., *et al*. (2010). Rational design of cationic lipids for siRNA delivery. *Nature Biotechnology*, *28*(2), 172-176. https://doi.org/10.1038/nbt.1602

Shi, Y., & Lammers, T. (2019). Combining nanomedicine and immunotherapy. *Accounts of Chemical Research*, *52*(6), 1543-1554. https://doi.org/10.1021/acs.accounts.9b00148

Svitkin, Y. V., Cheng, Y. M., Chakraborty, T., *et al*. (2017). N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms. *Nucleic Acids Research*, *45*(10), 6023-6036. https://doi.org/10.1093/nar/gkx135

Tenchov, R., Bird, R., Curtze, A. E., & Zhou, Q. (2021). Lipid nanoparticles-From liposomes to mRNA vaccine delivery. *ACS Nano*, *15*(11), 16982-17015. https://doi.org/10.1021/acsnano.1c04996

Tros de Ilarduya, C., Sun, Y., & Düzgüneş, N. (2010). Gene delivery by lipoplexes and polyplexes. *European Journal of Pharmaceutical Sciences*, *40*(3), 159-170. https://doi.org/10.1016/j.ejps.2010.03.019

Van Hoecke, L., Verbeke, R., Dewitte, H., *et al*. (2020). mRNA in cancer immunotherapy: Beyond a source of antigen. *Molecular Cancer*, *19*(1), 48. https://doi.org/10.1186/s12943-020-01163-7

Verbeke, R., Lentacker, I., De Smedt, S. C., & Dewitte, H. (2021). The dawn of mRNA vaccines: The COVID-19 case. *Journal of Controlled Release*, *333*, 511-520. https://doi.org/10.1016/j.jconrel.2021.03.043

Weng, Y., Li, C., Yang, T., *et al*. (2020). The challenge and prospect of mRNA therapeutics landscape. *Biotechnology Advances*, *40*, Article 107534. https://doi.org/10.1016/j.biotechadv.2020.107534

Wesselhoeft, R. A., Kowalski, P. S., Parker-Hale, F. C., *et al*. (2019). RNA circularization diminishes immunogenicity and can extend translation duration *in vivo*. *Molecular Cell*, *74*(3), 508-520. https://doi.org/10.1016/j.molcel.2019.02.015

Zeng, C., Zhang, C., Walker, P. G., & Dong, Y. (2022). Formulation and delivery technologies for mRNA vaccines. In *Current Topics in Microbiology and Immunology* (Vol. 440, pp. 71-110). Springer. https://doi.org/10.1007/82_2020_217.

Cite this article: Giri N, Maske U. Engineering mRNA Vaccines for Solid Tumors: Challenges in Delivery, Stability, and Clinical Translation. Asian J Biol Life Sci. 2027;XX(XX):1-14.