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REVIEW

Applications of mRNA Delivery in Cancer Immunotherapy

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Abstract: Cancer treatment is continually advancing, with immunotherapy gaining prominence as a standard modality that has markedly improved the management of various malignancies. Despite these advancements, the efficacy of immunotherapy remains variable, with certain cancers exhibiting limited response and patient outcomes differing considerably. Thus, enhancing the effectiveness of immunotherapy is imperative. A promising avenue is mRNA delivery, employing carriers such as liposomes, peptide nanoparticles, inorganic nanoparticles, and exosomes to introduce mRNA cargos encoding tumor antigens, immune-stimulatory, or immune-modulatory molecules into the tumor immune microenvironment (TIME). This method aims to activate the immune system to target and eradicate tumor cells. In this review, we introduce the characteristics and limitations of these carriers and summarize the application and mechanisms of currently prevalent cargos in mRNA-based tumor treatment. Additionally, given the significant clinical application of immune checkpoint inhibitors (ICIs) and chimeric antigen receptor (CAR)-based cell therapies in solid tumors (including melanoma, non-small-cell lung cancer, head and neck squamous cell carcinoma, triple-negative breast cancer, gastric cancer) and leukemia, which have become first-line treatments, we highlight and discuss recent progress in combining mRNA delivery with ICIs, CAR-T, CAR-NK, and CAR-macrophage therapies. This combination enhances the targeting capabilities and efficacy of ICIs and CAR-cell-based therapies, while also mitigating the long-term off-target toxicities associated with conventional methods. Finally, we analyze the limitations of current mRNA delivery systems, such as nuclease-induced mRNA instability, immunogenicity risks, complex carrier production, and knowledge gaps concerning dosing and safety. Addressing these challenges is crucial for unlocking the potential of mRNA in cancer immunotherapy. Overall, exploring mRNA delivery enriches our comprehension of cancer immunotherapy and holds promise for developing personalized and effective treatment strategies, potentially enhancing the immune responses of cancer patients and extending their survival time.

Keywords: mRNA immunotherapy, LNP-mRNA, CAR, ICI, tumor microenvironment modulation, personalized medicine

Introduction

Cancer treatment encompasses various therapeutic approaches, such as surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Currently, the application of concepts such as comprehensive treatment, precision medicine, and immunotherapy has made tumour treatment more personalized and effective.[1,2] Tumour immunotherapy is a therapeutic approach that stimulates or controls the immune system to target and destroy cancerous cells. Tumour immunotherapy encompasses various therapeutic approaches, including cancer vaccines, immune checkpoint inhibitors (ICIs) based on monoclonal antibodies, chimeric antigen receptor (CAR) T-cell immunotherapy, etc.[3] These therapeutic strategies aim to reactivate and sustain the tumour immune response, thereby restoring the body's intrinsic antitumour immune mechanisms and improving the management and eradication of cancers.

Despite significant progress in cancer treatment, several challenges and limitations are encountered in tumour immunotherapy. First, the efficacy of immunotherapy varies greatly among different types of cancer and even among patients with

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the same type of cancer, which is closely related to the genetic and molecular characteristics of the patients.[4] Immune escape is one of the main reasons for this.[5] Tumor cells exhibit high expression levels of programmed death-ligand 1 (PD-L1), which binds to programmed death-1 (PD-1) receptors on T cells, thereby inhibiting their tumor-cytotoxic functions.[6] The population of regulatory T cells (Tregs) also increases within the tumor microenvironment, where PD-1 on these cells interacts with PD-L1 on tumor cells or antigen-presenting cells, further enhancing immunosuppression.[7] Additionally, M2 macrophages secrete immunosuppressive cytokines that inhibit T cell activity and promote tumor angiogenesis.[8] Within the tumor-associated neutrophils, the N2 subtype releases reactive oxygen species and arginase, leading to damage of immune cells and depletion of arginine.[9] Collectively, these immune evasion mechanisms undermine the efficacy of immunotherapy to a certain extent. Second, in traditional immunotherapy, high doses of immune stimulants or antibodies are often used to activate the immune system. This method lacks specificity for particular tumour cells and may cause harm to normal tissues which may lead to severe adverse events.[10]

Messenger RNA (mRNA) delivery is a strategy that involves the direct administration of mRNA into cells to induce the production of specific proteins, thereby enabling targeted therapeutic intervention for a range of diseases. The mRNA molecules used in delivery can encode any protein required by humans, including tumour-associated antigens (TAAs), immunostimulatory factors, and immune regulatory molecules, thereby achieving the objectives of various therapies. Moreover, mRNA molecules demonstrate high safety, favorable degradability, and the advantage of not integrating into the genome, making them ideal carriers for therapeutic applications.[11,12]

In recent years, mRNA delivery systems have made significant strides in clinical development. The Pfizer-BioNTech and Moderna COVID-19 vaccines, which utilize this technology, received Emergency Use Authorization (EUA) and full approval from the Food and Drug Administration (FDA).[13] These vaccines employ lipid nanoparticles to deliver mRNA encoding the genetic information for the SARS-CoV-2 spike protein into human cells, thereby eliciting an immune response. Furthermore, Moderna's mRNA-1273 and BioNTech's BNT162b2 demonstrated outstanding efficacy in COVID-19 clinical trials.[14] These instances underscore the potential and reliability of mRNA delivery systems.

The methods of mRNA delivery technology mainly include nanotechnology and cell engineering.[15,16] Nanotechnology refers to the utilization of nanomaterials, such as liposomes, polymers, metals, and carbon, to encapsulate or load mRNA molecules, forming mRNA nanoparticles (NPs). These NPs can be administered into the human body via intravenous injection, intramuscular injection, nasal inhalation, or other routes to deliver mRNA into specific locations.[17,18] Cell engineering involves the transfection of mRNA molecules into ex vivo cultured human cells (such as dendritic cells (DCs), lymphocytes, and macrophages) through methods such as electroporation, gene guns, and viral vectors, followed by reinfusion of the transfected cells back into the human body.[16,19]

The application of mRNA delivery in tumour immunotherapy has significant advantages. First, through the personalized design and targeting of specific cancer types with mRNA sequences, a more effective immune response can be induced to improve treatment outcomes.[12] Second, mRNA delivery technology can efficiently and accurately deliver mRNAs encoding tumour-related antigens or other immune molecules into cells, triggering a specific immune attack on tumour cells. This precision allows mRNA delivery to identify and eliminate tumour cells effectively without damaging normal cells.[11,12] Additionally, compared with DNA vaccines, mRNA does not need to enter the nucleus for transcription, avoiding the potential risks associated with genomic integration, thus enhancing safety. It can also be translated rapidly in the cytoplasm, enabling a faster immune response.[20] In contrast to siRNA, mRNA has a broader range of applications. mRNA can encode a variety of antigens and immune-stimulating molecules, and it can customize personalized treatment plans according to different tumor types and the individual conditions of patients. However, siRNA is limited to silencing known disease-causing genes, and its applicability is poor when there are no clear disease-causing genes or when it is necessary to enhance the immune response.[21]

In the realm of tumor immunotherapy, numerous drug studies leveraging mRNA are being actively pursued, with many already in the clinical trial phase. Through in-depth exploration and meticulous curation on the authoritative platform ClinicalTrials.gov, we have selected several highly representative mRNA-based cancer treatment drugs, aiming to offer valuable references and insights for related research and applications (Table 1).

In this article, we present an overview of mRNA delivery strategies and the most commonly used carriers, including liposomes, peptide NPs, inorganic NPs, and exosomes. We discuss how researchers design mRNA constructs encoding diverse molecules, including tumour-associated antigens (TAAs), immune stimulatory factors, and immunoregulatory

Table 1 Representative Clinical Trials of mRNA-Based Cancer Therapeutics. All the Data in the Table are Sourced from ClinicalTrials.gov

Name	Delivery Platform	Disease	Administration Route	Clinicaltrials.gov Identifier	Phase
ABOD2011	Not available	Advanced solid tumors	Intratumoral injection	NCT05392699	Phase I
CVGBM	Not available	Glioblastoma	Intramuscular injection	NCT05938387	Phase I
iNeo-Vac-R01	Not available	Digestive System Neoplasms	Intrahilar injection	NCT06026774	Phase I
LCs	Langerhans-type dendritic cells	Melanoma	Electroporation	NCT01456104	Phase I
MTS105	Lipid Nanoparticle (LNP)	Advanced hepatocellular carcinoma	Intravenous injection	NCT06689540	Phase I
mRNA-2752	Lipid Nanoparticle (LNP)	Relapsed/refractory solid tumor malignancies or lymphoma	Intratumoral injection	NCT03739931	Phase I
mRNA tumor vaccines	Not available	Pancreatic Cancer	Not available	NCT06156267	Early Phase I
mRNA Neoantigen Vaccine	Not available	Non-Small-Cell Lung Cancer	Not available	NCT06735508	Early Phase I
Neoantigen mRNA Vaccine	Not available	Solid Tumor	Not available	NCT06195384	Phase I
BNT141	Not available	CLDN18.2-positive tumors	Intravenous injection	NCT04683939	Phase I/II
DC-006	Not available	Recurrent Epithelial Ovarian Cancer	Intradermal injection	NCT01334047	Phase I/II
pp65 RNA LP (DP1), pp65/ tumor mRNA RNA-LP (DP2)	Lipid Nanoparticle (LNP)	Recurrent Pulmonary Osteosarcoma, Recurrent High-grade Glioma	Not available	NCT05660408	Phase I/II
STX-001	Not available	Triple-negative breast cancer and melanoma	Intratumoral Injection	NCT06249048	Phase I/II
W_pro1/BNT112	Lipid Nanoparticle (LNP)	Prostate cancer	Intravenous injection	NCT04382898	Phase I/II
mRNA-4157	Lipid Nanoparticle (LNP)	Melanoma	Not available	NCT03897881	Phase II
mRNA-1273	Lipid Nanoparticle (LNP)	Solid tumor and blood cancer	Intramuscular injection	NCT04847050	Phase II
BNT162b2	Lipid Nanoparticle (LNP)	Hematopoietic Neoplasms	Intramuscular injection	NCT04951323	Phase III
mRNA Tumor Vaccine	Lipid Nanoparticle (LNP)	Esophageal Cancer,Non Small Cell Lung Cancer	Subcutaneous injection	NCT03908671	Not Applicable

molecules, to be delivered into the body for cancer treatment. Furthermore, we explore the synergistic potential of combining mRNA delivery with ICIs to enhance their efficacy in cancer treatment. Additionally, we examine the application of mRNA delivery in CAR cell therapy, focusing on how mRNA delivery enables transient CAR expression to improve the targeting ability and effectiveness of CAR-T, CAR-engineered natural killer (NK) (CAR-NK), and CAR-engineered macrophage (CAR-M) cells while overcoming the long-term off-target toxicity associated with traditional methods. Finally, we analyse the limitations of current mRNA delivery methods and propose future directions to enhance their role in immunotherapy.

mRNA Delivery Carriers

In mRNA delivery systems for cancer therapy, the selection of an appropriate delivery vehicle is crucial to ensure effective cellular uptake and achieve the desired therapeutic outcomes. This chapter provides a detailed exploration of several major

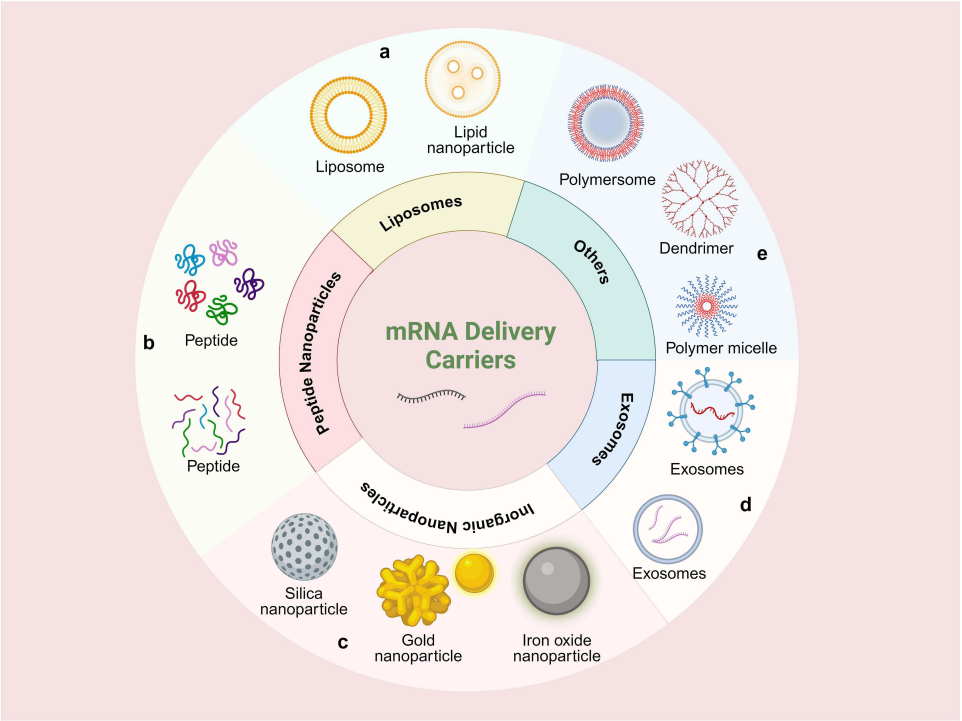

Figure 1 mRNA delivery carriers. mRNA delivery carriers include liposomes (**a**), peptide nanoparticles (**b**), inorganic nanoparticles (**c**), exosomes (**d**) and others (**e**). Created in BioRender. Dai, C. (2025) https://BioRender.com/u23s018.

delivery vehicles, including liposomes (Figure 1a), peptide NPs (Figure 1b), inorganic NPs (Figure 1c), exosomes (Figure 1d) and other carriers such as polymersomes, dendrimers, and polymer micelles (Figure 1e). Each of these carriers possesses distinct physicochemical properties and biocompatibility, enabling them to serve various therapeutic needs. Liposomes, being among the earliest delivery vehicles, have a structure similar to that of cell membranes, facilitating mRNA membrane fusion and internalization.[22,23] Peptide NPs and inorganic NPs are notable for their excellent stability and modifiable surface properties, showing broad application prospects in preclinical studies.[24,25] Owing to their natural biocompatibility and targeting capabilities, exosomes have emerged as promising delivery systems.[26] Additionally, novel carriers such as polymersomes, dendrimers, and polymer micelles are gaining attention because of their high controllability and targeting potential.[27,28] The following sections delve into the characteristics of these different mRNA delivery vehicles.

Liposomes

Liposomes are an early version of lipid NPs (LNPs), and they have become a widely used nanocarrier platform because of their ability to facilitate the transportation of both hydrophobic and hydrophilic molecules, including very small molecules as well as nucleic acids.[29] Liposomes are made up of several key components (Figure 2). Ionic lipids are essential because they form stable complexes with negatively charged mRNAs, facilitating their intracellular release. Helper lipids increase the structural stability of liposomes and aid in liposome fusion with cellular membranes. Cholesterol increases the stability and fluidity of liposomes, improving their formation and mRNA delivery efficiency. Finally, polyethylene glycol (PEG)-modified lipids create a "stealth layer" on the surface of liposomes, allowing them to evade immune system recognition and clearance. Compared with other delivery methods, the encapsulation of mRNA in liposomes increases stability and efficacy, improves delivery efficiency, and reduces unnecessary immune responses.[29,30]

In recent studies, liposomes have been further advanced as mRNA delivery vehicles through the surface modification of liposomes, enabling targeted delivery of mRNA to specific cells. For example, Joel et al modified the surface of liposomes with anti-CD5 antibodies, precisely targeting T cells and successfully reprogramming them in vivo.[31] Researchers injected Ai6 mice carrying the Rosa26CAG-LSL-ZsGreen gene with 30 μg of non-targeted/LNP-Cre (abbreviated as NT), IgG/LNP-

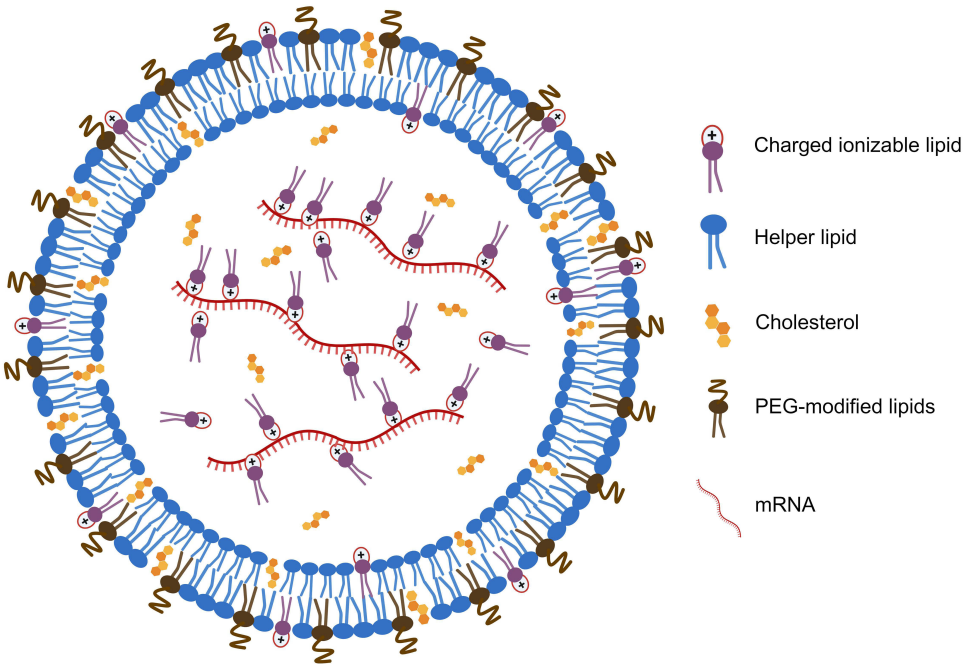

Figure 2 The key components of Liposomes. Liposomes consist of several key components, each with distinct functions. Charged ionizable lipids electrostatically interact with negatively charged mRNA, enabling its encapsulation into liposomes and promoting cell entry. Neutral helper lipids stabilize the bilayer structure, optimize morphology, and prevent aggregation. Cholesterol regulates membrane fluidity and ensures structural integrity during circulation and aids in cell contact. PEG-modified lipids form a hydrophilic surface layer, reducing immune recognition and clearance, prolonging circulation time, and enhancing delivery efficiency. Created in BioRender. Dai, C. (2025) https://BioRender.com/z14b486.

Cre, or CD5/LNP-Cre. After 24 hours, the expression of ZsGreen protein was observed in 81.1% of $CD4^+$ cells and 75.6% of $CD8^+$ cells. Farasat A et al demonstrated that oligoclonal NPs exhibit greater cytotoxicity towards HER2-positive tumour cells than nontargeted liposomes do. The oligoclonal targeting of liposomes is thus regarded as a promising strategy for treating breast cancer characterized by HER2 overexpression.[32] Additionally, Goswami R et al developed a panel of LNPs in which the surface is modified with mannan, revealing elevated levels of IgG1 and IgG2a in mannose-sylated lipid NPs (MLNPs) relative to nonmodified LNPs. This study confirmed the increased efficacy of mannosylated self-amplifying RNA (SAM) LNPs for the delivery of RNA vaccines compared with nonglycosylated delivery systems.[33] Current research directions include developing novel lipid materials to improve delivery efficiency and safety, designing targeted delivery systems to increase tissue specificity, and exploring the potential applications of liposomes in treating other diseases. As these technologies continue to advance, liposome-encapsulated mRNA delivery is expected to play a greater role in vaccine development, gene therapy, and other biopharmaceutical fields.

Peptide NPs

The formation of peptide NPs is based on the self-assembly process of peptide molecules, which spontaneously assemble into stable nanostructures via noncovalent interactions, including electrostatic interactions, hydrophobic interactions, and hydrogen bonding.[24] mRNA can be combined with self-assembled peptide NPs either by direct mixing or by being encapsulated within the particles during the peptide self-assembly process.[34] Peptide NPs can be designed to facilitate cellular uptake, thus improving delivery efficiency. Additionally, by incorporating specific cell-targeting signals into peptide sequences, targeted delivery to particular cell types or pathological tissues can be achieved.[35] Owing to its unique advantages and potential, the technique of encapsulating mRNAs within peptide NPs has become a research focus in the fields of gene therapy and vaccine development.

Biocompatibility is a crucial factor for any delivery system, and peptide nanoparticles excel in this regard. Their components are usually derived from natural or biocompatible synthetic peptides, which minimizes the possibility of triggering adverse immune responses when introduced into the body. This enables them to integrate more smoothly with

biological systems and reduces the risks of rejection or inflammation. Peptides have been widely explored for gene delivery purposes because of their ability to be synthesized as uniform single-molecule entities.[36–38] Peptides are inherently biodegradable and offer precise control over various properties, including charge distribution, hydrophobicity, and structural geometry.[36,39] Cell-penetrating polypeptides offer the added benefit of facilitating folding-dependent complexation, which allows them to shield nucleic acids through secondary and tertiary interactions, thereby protecting the nucleic acids from enzymatic breakdown. By adjusting these interactions, it is possible to reduce aggregation and achieve size distributions within a narrow range, with 50–300 nm typically considered optimal.[40,41] Furthermore, scientists have made it possible to design purely synthetic peptide sequences by investigating the cell-penetrating motifs associated with cellular uptake and their relationship with nucleic acid packaging.[42,43]

However, despite these advantages, the development of peptide NPs also faces several design challenges. One significant concern is their stability in vivo. Once administered, peptide NPs encounter a complex physiological environment that can potentially disrupt their nanostructure. For example, enzymatic degradation in the bloodstream or within cells may lead to the premature release or breakdown of the encapsulated mRNA. Moreover, changes in pH and ionic strength can also impact the noncovalent interactions that hold the peptide NPs together, affecting their overall stability and functionality.[44] Overcoming these challenges requires careful optimization of peptide sequences, chemical modifications, and formulation strategies to ensure that peptide NPs can maintain their integrity and effectively deliver mRNA to the target site.[45] With a deeper understanding of peptide design and nanotechnology, along with advances in production and purification techniques, this delivery system is expected to play a progressively significant role in the future of biomedicine.[46,47]

Inorganic NPs

Inorganic NPs mainly include nonmetal NPs, metal NPs, and oxide NPs, among other types.[25] Among the most representative NPs are silica NPs, which have been extensively studied for use in mRNA delivery because of their unique physicochemical properties and biocompatibility.[48] As an inorganic material, silica offers several advantages. First, the high surface area of silica enables the loading of a larger amount of mRNA molecules.[49] Second, the release rate of mRNA can be controlled by adjusting the pore structure of the silica NPs, enabling controlled release during delivery.[50] Third, surface modifications (eg, covalent bonding of targeting ligands) can improve the ability of silica NPs to target specific cell types or pathological tissues. Moreover, silica is degradable and biocompatible.[51] Although silica NP-encapsulated mRNA delivery technology has shown tremendous application prospects, further research is needed to determine the in vivo distribution and clearance of silica NPs. Improper distribution may lead to accumulation in nonspecific tissues, whereas inefficient clearance could pose long-term biosafety issues. The future holds the promise of existing challenges being overcome to achieve safer, more effective, and smarter nucleic acid delivery solutions.[52,53]

The use of metal-core NPs as targeted delivery carriers has gained increasing popularity because of their ability to shield RNA payloads and direct them to their intended destinations.[54,55] The biological barriers present in vivo necessitate size requirements for drug delivery carriers; to evade recognition by the human immune system and enter cellular environments, carriers must be very small (<100 nm). Iron oxide and gold-core NPs can be synthesized under highly controlled conditions to produce uniform, ultrasmall drug delivery carriers capable of circumventing critical biological barriers. Additionally, unlike virus-based transfection reagents, the biodistribution of metal-core NPs can be monitored via noninvasive imaging techniques that leverage the interaction between electromagnetic radiation and the inorganic atoms of the NP core.[56] Furthermore, metal-core NPs have demonstrated transfection efficiencies comparable to those of traditional RNA delivery carriers while having the advantages of lower immunogenicity and minimal side effects, especially when tumour-targeting ligands are conjugated to their surfaces.[57,58]

Exosomes

Exosomes are extracellular vesicles containing components of the cells that secrete them, such as DNA and RNA. These exosomes can be absorbed by distant cells, thereby influencing the functions and behaviours of the absorbing cells.[26] The advantage of the use of exosomes as mRNA delivery vehicles lies in their natural origin and biological activity. Derived from their ability to be secreted by cells, exosomes possess low immunogenicity and toxicity, thereby reducing the risk of

side effects during the treatment process.[59] Furthermore, the surface of exosomes is enriched with proteins, lipids, carbohydrates, and other biomolecules, which can serve as recognition signals to guide the precise targeting of exosomes to specific cells or tissues.[60] During the process of mRNA delivery, exosomes protect mRNAs from degradation and promote their cellular uptake. The double-layer membrane structure of exosomes provides a stable protective shell for mRNAs, preventing their enzymatic degradation in vivo. Simultaneously, exosomes release mRNAs into target cells through membrane fusion, achieving efficient and safe delivery. Additionally, exosomes exhibit excellent drug-loading capacity.[61] Scientists can modify the surface molecules or alter the internal environment of exosomes to load different types and quantities of drugs. This modification can enable exosomes to carry other therapeutic molecules alongside mRNA, facilitating multifunctional combinations.

However, despite these remarkable advantages, the scalability of exosome production remains a significant hurdle for clinical translation. Currently, the isolation and purification methods of exosomes are complex, time-consuming, and often yield relatively low amounts of exosomes. For example, ultracentrifugation, a commonly used isolation technique, requires expensive equipment and skilled operators, and the process is not easily scalable.[62] Alternative methods like size-exclusion chromatography and immunoaffinity capture also have their own limitations in terms of throughput and cost.[63] Moreover, even if exosomes can be produced in larger quantities, ensuring the consistency and quality of each batch becomes another challenge. Variations in the source cells, culture conditions, and isolation procedures can all lead to differences in exosome properties, which may affect their performance as mRNA delivery vehicles.[64,65] Tackling these scalability issues demands the development of innovative and more efficient production, isolation, and quality control strategies to make exosome-based therapies a viable option in the clinic. In conclusion, exosomes possess unique advantages and potential as mRNA delivery vehicles. The natural ability of exosomes to achieve efficient and safe mRNA delivery provides exciting new possibilities in the development of cancer immunotherapy.[66,67]

Others

In addition to the carriers mentioned above, many other carriers also have high application potential. Polymersomes, dendrimers, and polymer micelles are widely studied polymeric nanocarriers that are primarily used in drug delivery systems. These nanocarriers have garnered significant attention from the scientific community because of their unique properties and potential applications. Polymersomes are vesicular structures formed by the self-assembly of amphiphilic block copolymers, and they resemble liposomes in their structure.[27] Polymersomes consist of a bilayer polymer membrane that can encapsulate and protect drugs, thereby increasing drug stability. Compared with traditional liposomes, polymersomes exhibit increased stability and tunable permeability, making them highly useful in drug delivery systems.[68,69] Polymersomes can encapsulate both hydrophobic and hydrophilic drugs, and their surfaces can be modified for targeted delivery and controlled release.

Dendrimers are highly branched macromolecules that typically originate from a core molecule and are constructed by sequential chemical reactions that add hierarchical branching structures. The size, shape, and surface functionality of dendrimers can be precisely controlled. Owing to their high loading capacity and ease of modification, dendrimers are ideal drug delivery carriers, enabling targeted delivery through multiple functional groups on their surface.[70–72] One of the most remarkable unique advantages of dendrimers lies in their extremely high surface area to volume ratio. This characteristic enables them to carry a large number of functional groups, not only facilitating their modification with various ligands for targeted delivery but also allowing for enhanced drug loading.[73] For instance, dendrimers can be conjugated with specific antibodies or peptides that recognize and bind to receptors overexpressed on tumor cells, achieving precise tumor-targeted drug delivery.[74] Moreover, their unique structure endows them with the ability to penetrate biological membranes more effectively compared to other carriers.[75] This is particularly beneficial in applications such as gene therapy, where efficient intracellular delivery of nucleic acids is crucial. In addition to drug and gene delivery, dendrimers' functionalization potential extends to other biomedical fields. They can be engineered to serve as imaging agents, by incorporating fluorescent or radioactive substances, enabling real-time monitoring of drug distribution and therapeutic efficacy in vivo.[76] Their versatility and multi-functionality make them a promising candidate in modern biomedical research.

Polymer micelles are nanoscale micellar structures formed by the self-assembly of amphiphilic block copolymers. Polymer micelles consist of a hydrophobic core and a hydrophilic shell, allowing them to remain stable in aqueous

environments. These nanocarriers provide effective solubilization and protection of drugs, especially hydrophobic drugs. Polymer micelles are commonly used for the delivery of poorly soluble drugs, and their structure can be adjusted to achieve sustained release and targeted delivery.[28,77,78] In summary, as advanced drug delivery carriers, polymersomes, dendrimers, and polymer micelles offer new possibilities for increasing drug bioavailability, stability, and efficacy. With the advancement of nanotechnology, these nanocarriers are expected to be more widely applied in mRNA delivery and to play a significant role in clinical cancer therapy in the future.

mRNA Delivery Cargos Related to Cancer Immunotherapy

mRNAs, as versatile therapeutic tools, can increase the ability of the immune system to target cancer by encoding various biomolecules (Figure 3). Once delivered into the body, mRNAs can encode tumour-associated antigens, thereby promoting recognition by the immune system and the attack of cancer cells—a strategy commonly employed in mRNA vaccines.[79] Additionally, mRNAs can encode immunostimulatory molecules such as interleukins (ILs) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which effectively activate and improve the functionality of immune cells, increasing their cytotoxicity against cancer cells.[48,80] Moreover, mRNAs can also encode immunomodulatory molecules to further optimize the immune response, ensuring the effectiveness and durability of the immune system's fight against cancer.[81] By delivering mRNAs that encode different molecules, we can achieve more precise and potent therapeutic effects in cancer immunotherapy.

Tumour-Associated Antigens

Tumour-associated antigens (TAAs) are antigen molecules present on both tumour and normal cells that are highly expressed during tumour cell proliferation.[82] The use of mRNA vaccines to carry TAAs to activate the immune system has become a feasible approach for cancer treatment.[83] An mRNA vaccine is a particular type of vaccine in which mRNA is used to transmit genetic instructions that encode TAAs.[84] For example, in one study, mRNA vaccines were used to deliver TAAs to antigen-presenting cells (APCs), which include DCs. After taking up the mRNA vaccines encoding TAAs, DCs process TAAs into antigenic peptides and display them on MHC class I molecules. $CD8^+$ T cells recognize these complexes through TCRs, which is the primary signal for activation. After activation, $CD8^+$ T cells differentiate

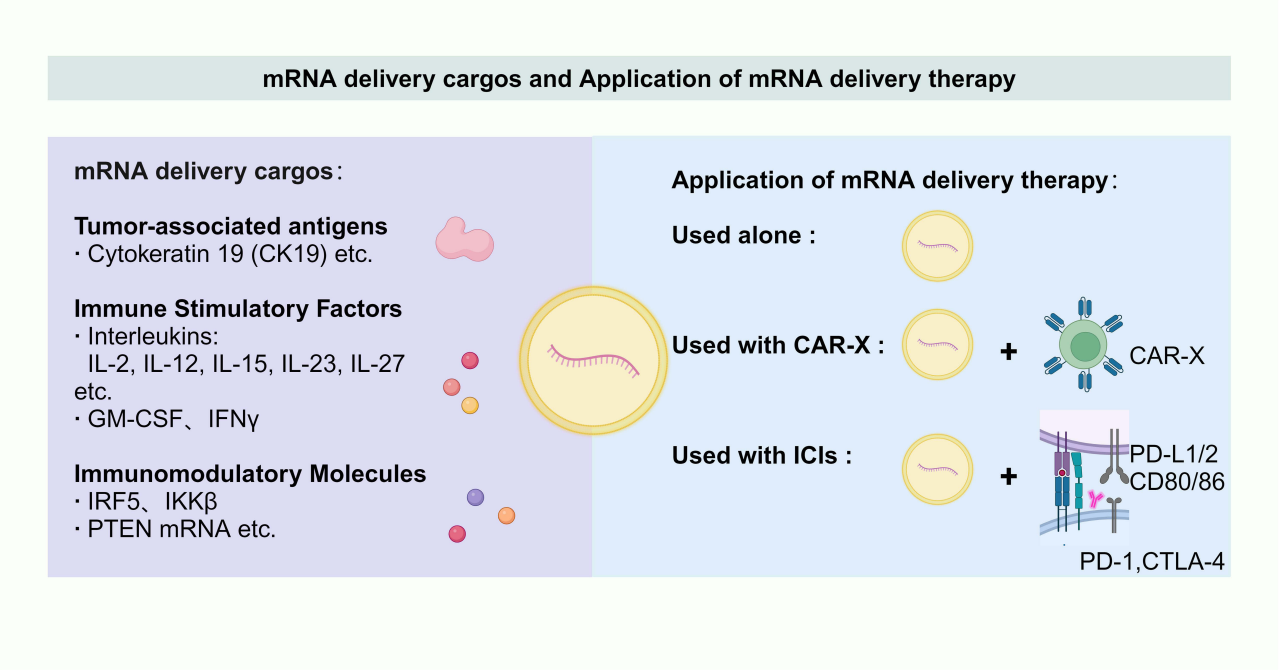

Figure 3 mRNA delivery cargos and applications of mRNA delivery therapy. Created in BioRender. Dai, C. (2025) https://BioRender.com/l82a560.

into CTLs, which can kill cancer cells expressing specific TAAs.[85] The co-stimulatory signals (the second signal) provided by molecules such as CD80 and CD86 on DCs work in concert with antigen recognition to enhance the activation of $CD8^+$ T cells.[86] The interaction between the two forms the backbone of anti-tumor immunity and enables the targeted attack on cancer cells. Additionally, CD4+ T cells can simultaneously activate antigen-specific B cells, inducing an immunological response in the body's fluids and achieving a sustained therapeutic reaction due to immune memory.[87] Currently, cancer immunotherapy targeting TAAs has entered a popular phase, with TAAs being among the leading research targets in solid tumour treatments.[79] Cytokeratin 19 (CK19) is a protein distributed on the cell membrane, and it is also commonly found in lung cancer cells, where it serves as a specific antigen for immune therapy.[88,89] In one study, the authors designed a cationic liposome-protamine complex (LPC) for delivering mRNA encoding CK19, thereby triggering an immune response that targets and inhibits the growth of tumours. The results showed that LPC/mRNA efficiently facilitated the internalization of antigens by DCs, induced the maturation of DCs, and augmented the release of cytokines, thus eliciting an inner-cell immunological response. Overall, this study revealed that LPC/mCK19 is a promising mRNA delivery therapeutic.[90]

In addition to presenting TAAs to APCs, mRNA vaccines require additional activation signals for APCs to be effective. The efficacy of mRNA vaccine administration relies on the uptake of mRNAs by APCs through phagocytosis and the ability of mRNAs to stimulate the maturation of APCs. In one study, Sandra van Lint et al used codelivery of TAA mRNA with TriMix (a mixture of mRNAs encoding a CD40 ligand, constitutively active Toll-like receptor 4, and CD70), and the results showed that this method induced an environment that attracted and stimulated T cells, including the process of enlisting CD4+ and CD8+ T cells, as well as cytotoxic T lymphocytes (CTLs), that specifically target different TAAs.[91]

In a study by Chen et al, the authors investigated the use of LNPs for targeting lymph nodes in the delivery of mRNA cancer vaccines. The authors demonstrated that this approach effectively directs the mRNA vaccine to lymph nodes, leading to robust activation of CD8+ T cells. LNP-mediated delivery significantly improves the immune response by inducing a strong CD8+ T-cell response against cancer cells. This targeting strategy not only increases vaccine efficacy but also optimizes immune activation in lymphoid tissues, which is crucial for generating a potent antitumour immune response. The findings of this study underscore the potential of LNP-based mRNA vaccines in advancing cancer immunotherapy by effectively leveraging lymph node targeting to boost immune responses.[92]

The method of administering mRNA tumour vaccines is a critical factor in ensuring their effectiveness and safety. Depending on the specific type of vaccine and the treatment goals, various administration methods can be chosen, such as subcutaneous injection, intravenous injection, and intramuscular injection.[93–95] Administration through the transfection of DCs is also a promising approach that can leverage the crucial role of DCs in the immune system to achieve targeted delivery and efficient expression of the vaccine in the body.[96] For example, a Phase 1 trial was conducted to evaluate the effectiveness of monocyte-derived DCs that were exposed to tumour RNA in children and young adults with brain cancer. Additionally, nasal administration has been proposed as a potentially effective vaccination strategy.[97]

Immune Stimulatory Factors

Immune stimulatory factors, such as ILs, interferons (IFNs), tumour necrosis factors (TNFs), and CSFs, are a group of molecules capable of activating and improving the body's immune response. By interacting with cells or molecules within the immune system, immune stimulatory factors trigger the activation and multiplication of immune cells, thereby improving the body's immunological response.[98] In the exploration of new strategies for cancer immunotherapy, the use of mRNAs encoding immune stimulatory factors as therapeutic agents has emerged as a focal area of interest. The introduction of mRNAs encoding specific immune stimulatory factors into the body enables the intracellular expression and release of the corresponding cytokines. These immune stimulatory factors can bind to receptors on immune cells, promoting the activation and proliferation of these cells and thus improving the body's ability to recognize and eliminate tumour cells.

Interleukins

The IL family comprises a collection of cytokines that are synthesized by multiple cells, and these ILs bind to specific receptors, transmit information, and activate immune cells. Among them, IL-2, IL-12, IL27, IL-15, and IL23, as well as others, have been widely studied and applied to immunotherapy for cancer.

IL-2 was one of the earliest ILs discovered. Through its stimulatory and activating effects on effector lymphocytes, IL-2 plays an essential role in immune homeostasis.[99] By delivering IL-2 mRNA, the immune system can be activated to target and destroy cancerous cells. A previous study described the progress of the delivery of tumour-activated IL-2 mRNA by LNPs. The researchers prepared ionizable lipid U-101-derived NPs (U-101-LNPs), and through in vitro transcription, they designed and synthesized IL-2F mRNA encoding a fusion protein composed of the linker IL-2 and CD25 (IL-2Rα). The results showed that the U-101-LNP/IL-2F mRNA formulation exhibited effective antitumour activity and safety, demonstrating its potential applicability in clinical tumour immunotherapy.[100] In another study, intratumoral injection of porous silica NP (PSNP) IL-2 mRNA into mouse tumour models stimulated immunogenic cancer cell death in tumour-bearing mice, reflecting the potential application of IL-2 mRNA delivery in tumour immunotherapy.[48]

IL-12 is a powerful immunostimulatory factor that can activate NK cells and T cells, inducing a strong antitumour effect.[101] In a MYC oncogene-driven hepatocellular carcinoma model, IL-12 mRNA LNPs exhibited significant antitumour effects. Treatment with IL-12-LNPs significantly reduced the liver tumour burden, delayed tumour progression, and increased survival rates in MYC-induced hepatocellular carcinoma (HCC) transgenic mice. By activating and increasing the activity of immune cells, such as by increasing the infiltration of immune cells, such as helper CD3+CD4+ T cells, into the tumour, IL-12-LNPs effectively suppressed tumour growth and metastasis.[102] IL27 is also an important research direction in cancer studies.[103] Researchers have used LNPs as a delivery vehicle to directly administer mRNAs encoding IL-12 and IL-27 into tumours, leading to the death of tumour cells and the suppression of tumour expansion. Furthermore, the combined use of IL-12 and IL-27 mRNAs further improved the therapeutic effect.[104]

Di Trani CA et al investigated the antitumour effects of intratumoral injection of IL-12-encoding mRNAs in Ringer's lactate solution. The researchers verified mRNA expression in tumour tissues by injecting luciferase (LUC)-encoding mRNAs. The in vivo results demonstrated that different doses of IL-12 mRNA (ranging from 10 μg to 0.5 μg) significantly increased IL-12p70 levels in tumour tissue compared with those in control tissue. In the B16-OVA tumour model, local injection of IL-12 mRNA at various doses had pronounced antitumour effects, with tumours in the treated sites completely disappearing in at least 50% of the mice and notable suppression of distant untreated tumours. All three doses (10 μg, 6 μg, and 0.5 μg) of IL-12 mRNA significantly increased overall survival, with the three-injection regimen being more effective than the single- or double-injection regimens. Overall, even the lowest dose of 0.5 μg of mRNA achieved effective antitumour outcomes.[105]

IL-15 has various functions, including determining T-cell responses and activating NK cells.[106] By delivering IL-15 mRNA, the antitumour activity of T cells can be improved. In one study, researchers prepared a cationic liposome–protamine complex system (CLPP) that efficiently delivered mRNA encoding IL-15 to C26 cells. The CLPP/mIL-15 complex, whether administered locally or systemically, effectively suppressed the growth of tumours in various C26 mouse colon cancer models.[107] A group of researchers have developed a nanoplatform called DPPA/IL-15 NPs. This nanoplatform was designed to specifically target PD-L1 in tumours and deliver IL-15 mRNA. The release of IL-15 mRNA resulted in a powerful immune response throughout the body and effectively suppressed tumour growth.[108]

Christian Hotz et al investigated the therapeutic potential of intratumoral injection of a cytokine–mRNA mixture, including IL-12sc, GM-CSF, IFN-α, and IL-15 sushi, for antitumour activity. The researchers reported that repeated injections of this cytokine–mRNA mixture led to substantial control of tumour growth and complete regression in the majority of B16F10 and CT26 tumour-bearing mice. A single injection was also effective in controlling tumour growth in the B16F10 model mice. The mRNA-encoded cytokines were expressed intratumorally and increased serum cytokine levels. In vivo experiments confirmed that the four-component mixture had superior antitumour effects and overall survival compared with single cytokine mRNA treatments, with complete tumour regression observed in 6 of 10 mice treated with the full cytokine mixture. Additionally, successful treatment led to resistance to tumour rechallenge, suggesting the development of immunologic memory. Antibody-mediated depletion studies showed that CD8+ T cells were the most critical for antitumour effects, followed by CD4+ T cells and NK cells.[109]

In addition, in a previous study, Hewitt SL, Bai A, and others provided a detailed introduction on how the cytokines IL-23, IL-36γ, and OX40L interact with immune cells and affect them to promote antitumour immune responses when encoded by mRNAs and delivered to the tumour microenvironment (TME).[110] Currently, an increasing number of ILs are being used in antitumour immune research, which not only demonstrates the potential of IL mRNA delivery-based cancer therapy but also provides a direction for future anticancer treatments.

However, it is crucial to note the potential side effects associated with interleukin - based immunotherapies. One significant concern is the occurrence of inflammatory cytokine storms. When interleukins are delivered or over - expressed during immunotherapy, they can lead to an excessive activation of immune cells. These over - activated immune cells then initiate a cascade of cytokine release.[111] Normally, the body has regulatory mechanisms in place to control cytokine levels. But with the potent stimulatory effect of certain interleukins in immunotherapies, this balance can be disrupted. The massive release of cytokines overwhelms the body's normal regulatory systems, potentially leading to life - threatening conditions such as systemic inflammation, fever, organ damage, and other severe symptoms.[112] To alleviate these adverse effects, dose modulation represents a direct and efficacious strategy. By meticulously adjusting the dosage of delivered interleukin - related mRNA, an optimal equilibrium can be achieved between attaining potent antitumour efficacy and minimizing the potential risk of cytokine storms. Another viable approach entails the combination of interleukins with other pharmacologic agents. For instance, certain drugs capable of modulating the immune response, such as corticosteroids or specific monoclonal antibodies, can be administered concomitantly.[113] This combinatorial regimen serves to attenuate the excessive immune activation instigated by interleukins.

GM-CSF and IFN-γ

GM-CSF is a crucial cytokine that is involved primarily in stimulating and regulating the generation, differentiation, and function of granulocytes and macrophages.[114] Currently, GM-CSF mRNA can be injected into the body together with mRNA vaccines as a component to increase the immunogenicity of tumour vaccines. In one study, researchers used SV40 large T antigen (TAg) as a model tumour/autoantigen, and the experimental results in transgenic mouse prostate cancer (TRAMP) demonstrated that GM-CSF increased the ability of mRNA vaccines to treat established spontaneous tumours.[80] GM-CSF mRNA can also be used in combination with cytokine-encoded mRNAs to increase the efficacy of tumour immunotherapy. In a previous study, Liu et al used cytokine-encoded mRNA-loaded LNPs for anticancer therapy, and the results showed that GM-CSF mRNA exhibited a synergistic effect with cytokine-encoded mRNA. The combined use of GM-CSF mRNA with IL12 and IL27 mRNAs improved the anticancer therapeutic effect.[104] When it comes to the delivery of GM-CSF mRNA, the choice of delivery vehicle plays a significant role. LNPs can protect GM-CSF mRNA from degradation by encapsulating it, thus enhancing its stability during in vivo transport. Their size and surface properties can also influence the bioavailability of GM-CSF. Smaller LNPs with appropriate surface modifications may facilitate better cellular uptake, leading to more efficient translation of GM-CSF mRNA and increased local and systemic bioavailability of the cytokine.[115]

IFN-γ plays a crucial role in the activation of cellular immunity and the subsequent stimulation of antitumour immune responses.[116] Researchers have demonstrated, using IFN-γ as an example, that the cell-enhancing RNA system (CERS) mediates mRNA therapy to induce an approximately 40% increase in translation efficiency and captures approximately 40% of unstable IFN-γ protein, resulting in a doubled duration of IFN-γ activity. Regarding the delivery aspect, CERS not only improves the translation efficiency but also impacts the stability and bioavailability of IFN-γ. The unique structure of CERS provides a protected microenvironment for IFN-γ mRNA, preventing its premature degradation. Moreover, the CERS and cells promotes the internalization of IFN-γ mRNA, enhancing its bioavailability. When applied to immunotherapy for osteosarcoma, CERS-mediated IFN-γ mRNA delivery effectively reprogrammed tumour-associated macrophages (TAMs) into tumoricidal macrophages and significantly inhibited tumour growth through the induction of antitumour immunity. Notably, CERS-mediated IFN-γ mRNA delivery combined with PD-L1 monoclonal antibody (αPD-L1) therapy remarkably led to the disappearance of highly malignant pulmonary metastases.[117]

There are also some potential immunostimulatory molecules that can be used for mRNA delivery to promote immunotherapy. Chemokines such as CXC chemokine ligand (CXCL9/10) and tumor necrosis factor-α (TNFα) have emerged as highly promising research directions in the future. Firstly, CXCL9/10, belonging to the chemokine family, can recruit and activate cytotoxic T lymphocytes and natural killer cells to the tumor site, enhancing the anti-tumor immune response.[118] When delivered via mRNA, their sustained local expression in the tumor is expected to improve the infiltration and killing effect of immune cells. Secondly, TNFα is capable of activating macrophages and enhancing their cytotoxicity to directly attack cancer cells.[119] It can also induce apoptosis of tumor cells. In combination with mRNA delivery technology, it may enhance its local effect at the tumor site while reducing systemic side effects. Current studies

have provided preliminary evidence of their potential, and further investigations are warranted to fully explore their therapeutic applications in cancer treatment.

Immunomodulatory Molecules

Under normal circumstances, the primary function of immune cells is to recognize and attack pathogens and abnormal cells,[120] thereby inhibiting the growth and spread of tumours. However, in certain special circumstances, immune cells may also promote tumour development. The delivery of mRNAs encoding immunoregulatory molecules to suppress the protumour effects of immune cells offers a new strategy in the fight against cancer.

Tumour-associated macrophages (TAMs) constitute one of the primary types of immune cells infiltrating tumours, constituting 30–50% of the stromal cells in the TME.[121] TAMs are generally classified into two functionally distinct subtypes: classically activated M1 macrophages and alternatively activated M2 macrophages. M1 macrophages typically exert antitumour effects by directly mediating cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) to kill tumour cells. In contrast, M2 macrophages can promote tumorigenesis and metastasis, suppress T-cell-mediated antitumour immune responses, increase tumour angiogenesis, and thereby contribute to tumour progression.[122] TAMs display an immunosuppressive M2-like phenotype in advanced malignancies. TAMs play pivotal roles in promoting tumour development, invasion, migration, angiogenesis, and immune suppression.[123] Therefore, TAM-targeted therapies are particularly important in anticancer strategies. By modulating or eliminating the immunosuppressive functions of TAMs, the ability of immune cells to attack tumour cells can be restored, thereby inhibiting tumour growth and spread. The application of TAMs as targets for cancer treatment holds promise for overcoming the limitations of traditional tumour therapy methods and achieving significant therapeutic effects.[124]

Reprogramming these TAMs to the M1 phenotype may inhibit their protumorigenic activities and unleash antitumour immunity. To explore this therapeutic strategy, researchers are utilizing nanomedicine carriers to deliver specific molecules or signals into TAMs, reprogramming their function towards an immune-activating state. These molecules can activate the antitumour functions of TAMs, promoting their attack on tumour cells and improving the overall immune response.[81] In previous studies, F. Zhang, N.N. Parayath, and others utilized nanocarriers as delivery systems for mRNAs. In ovarian cancer, melanoma, and glioblastoma models, infusion of NPs containing IRF5-encoding mRNA and its activating kinase IKKβ could reverse the immunosuppression and tumour-supportive state of TAMs and reprogramme them into a phenotype that could induce antitumour immunity and promote tumour regression. These genetically programmed macrophages could perform antitumour functions. In addition, this strategy increased the infiltration and activation of immune cells in the TME, improving the overall immunotherapy effect.[125] Michelle Seif et al introduced a novel method in which recombinant *Saccharomyces cerevisiae* is used as a gene delivery vehicle for primary human macrophages. The opsonized yeast was efficiently internalized by M2 macrophages, facilitating effective mRNA delivery and initiating the expression of proinflammatory cytokines. Notably, the expression of the protein encoded by the delivered mRNA was greater in M2 macrophages than in M1 macrophages. Moreover, delivering mRNA encoding the proinflammatory regulators MYD88 and TNF to M2 macrophages led to a sustained increase in proinflammatory and cytotoxic cytokines, indicating successful reprogramming of M2 macrophages towards the antitumour M1 phenotype. This study highlights that yeast-based gene delivery is a promising approach for treating certain conditions, such as cancer, that could benefit from M1-polarized macrophages.[126]

In the reprogramming of tumor-associated macrophages (TAMs), numerous challenges remain in targeting TAMs in solid tumors. The complex anatomical structure of solid tumors presents a physical barrier due to their dense stroma and chaotic vasculature, impeding the efficient delivery of mRNA-loaded carriers to TAMs. For instance, lipid nanoparticles (LNPs) struggle to penetrate deep into the tumor core.[127] The plasticity and heterogeneity of TAMs pose difficulties as well. Different solid tumors can induce diverse TAM phenotypes.[128,129] Soluble factors such as interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF) secreted by tumors can inhibit TAM activation and nullify reprogramming signals.[130,131] Overcoming these obstacles is crucial for effective mRNA-based cancer treatment.

Regulatory T (Treg) cells play crucial roles in maintaining immune tolerance and preventing autoimmune diseases. However, in the TME, Treg cells typically assist tumours in evading immune surveillance by suppressing effective immune responses.[132] Compared with reprogramming TAM strategies, strategies for reprogramming Treg cells into T effector cells via

NP drugs have been reported in only a limited number of studies.[133] Thus, further research into mRNA delivery therapies that target Treg cells to improve anticancer immune responses is warranted. Key receptors involved in the immunosuppressive functions of Treg cells include cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), a critical inhibitory receptor on Treg cells that suppresses effector T-cell activation by binding to CD80/CD86, thereby competitively inhibiting CD28-mediated activation signals.[134] CTLA-4 binding leads to the secretion of suppressive cytokines (eg, IL-10 and TGF-β) and reduces the activation and proliferation of effector T cells. Nrp1, a transmembrane glycoprotein specifically expressed on Treg cells, interacts with vascular endothelial growth factor (VEGF) and neurogenic molecules to influence Treg cell stability and function. Studies have demonstrated that mixed NPs targeting Treg cells (tLyp1-hNPs), decorated with tLyp1 peptide and carrying an anti-CTLA-4 ICI, effectively inhibit Treg suppressive functions and improve CD8+ T-cell antitumour effects.[135] IL-2R (particularly its α chain CD25) is highly expressed on Treg cells and is a key marker of their function and stability. IL-2 promotes Treg cell survival and proliferation by binding to IL-2R.[136] Additionally, receptors such as glucocorticoid-induced TNFR-related protein (GITR) and lymphocyte activation gene-3 (LAG-3) also contribute to maintaining Treg cell function.[137] We propose designing mRNA constructs encoding proteins that target these receptors to potentially alter their protumour effects. Additionally, myeloid-derived suppressor cells (MDSCs) are another type of protumour cell that can serve as a target for cancer therapy.[138]

mRNA Delivery Combined with ICIs

Immune checkpoints are regulatory pathways in the immune system that are essential for preserving self-tolerance and regulating the length and intensity of physiological immunological responses.[139] Tumour cells produce chemicals that stimulate these immunological checkpoints. Upon activation, antigens can no longer be presented to T cells, blocking the antigen presentation process in tumour immunity and thus suppressing the immunological activity of T cells. This enables tumour cells to evade detection and persist.[140,141] The most common immune checkpoint molecules include PD-1, CTLA-4, Lymphocyte Activation Gene-3 (LAG3), and T-cell immunoglobulin mucin receptor 3 (TIM3).[142–144] Currently, ICIs are established as first-line treatment options for a variety of solid tumors, including melanoma,[145] non-small-cell lung cancer,[146] head and neck squamous cell carcinoma,[147] triple-negative breast cancer,[148] and advanced gastric cancer.[149] Nonetheless, a significant proportion of patients do not experience tumor progression cessation. This is primarily due to chronic inflammation or immunosuppressive signals within the tumor immune microenvironment, which promote tumor immune escape and lead to resistance to ICI therapy.

Perez-Nunez et al reported that an increase in the expression level of a protein called ligand-dependent corepressor (LCOR) can disrupt tumour immune escape. The overexpression of LCOR makes cancer cells “visible” to the immune system, thereby generating an antitumour effect. Consequently, the team utilized extracellular vesicles to encapsulate LCOR mRNA and combined this with anti-PD-L1 treatment. The results showed that the combination of extracellular vesicle) LCOR mRNA therapy and anti-PD-L1 therapy overcame drug resistance and eradicated breast cancer metastasis in preclinical models.[150]

In mouse models, mutations or deletions of certain tumour suppressor genes, such as phosphatase and tensin homologue (PTEN), can lead to inadequate response or resistance to immune checkpoint blockade (ICB) therapy.[151,152] PTEN is a critical tumour suppressor gene with significant roles in tumour suppression and the regulation of immune responses.[153] Lin et al developed a new polymeric NP platform comprising mPEG-PLGA and the cationic lipid-like material G0-C14. In vivo experiments demonstrated that PTEN mRNA NPs have the ability to counteract the immunosuppressive TME by facilitating the infiltration of CD8+ T cells into tumour tissues, increasing the production of proinflammatory cytokines (such as IL-12, TNF-α, and IFN-γ), and decreasing the presence of T cells and MDSCs. Compared with treatment with anti-PD-1 alone, combining PTEN mRNA NPs with PD-1 antibodies achieved significant antitumour efficacy in mouse models of PTEN-mutant melanoma and PTEN-deleted prostate cancer.[154]

In the study by Li et al, the authors investigated the effects of mRNA lipid NP-mediated pyroptosis on “cold” tumours and its combination with ICIs. ‘Cold’ tumours are characterized by a lack of pre-existing immune infiltration, which means there are few immune cells actively engaging with the tumour cells. This paucity of immune cell activity is often due to immunosuppressive factors secreted by the tumour itself or the surrounding microenvironment. These factors can inhibit the recruitment and activation of immune cells, leading to a state where the tumour remains undetected or

unchallenged by the immune system. Research has shown that mRNAs delivered by LNPs encode proteins that induce pyroptosis in tumour cells. This form of cell death not only effectively activates tumour-specific immune responses but also promotes immune activation of the TME. This transformation makes originally "cold" tumours, which are less responsive to ICIs, more "hot" and significantly increases their sensitivity to ICIs. The combination of this pyroptosis-inducing strategy with ICIs improves the overall antitumour response, thereby improving therapeutic outcomes. This study provided a novel solution to the common issue of resistance in immunotherapy, and its findings highlighted the significant potential of combining mRNA delivery therapies with ICIs to increase the efficacy of cancer immunotherapy.[155]

In light of the potential involvement of p53 dysfunction in immunosuppression, scientists have conducted studies to examine the effects of reintroducing p53 expression to the TME and the efficacy of ICB. Researchers have developed and optimized an mRNA NP platform targeting CXCR4 to efficiently stimulate p53 expression in HCC models. In both orthotopic and ectopic p53-null HCC mouse models, the combination of CXCR4-targeted p53 mRNA NPs and anti-PD-1 therapy achieved better antitumour effects than did anti-PD-1 treatment alone. These findings suggest that the use of p53 mRNA nanomedicines in conjunction with ICB can effectively counteract immunosuppression in HCC and facilitate the adoption of this approach in cancer therapy.[156] In another study, researchers created NPs to deliver mRNA vaccines encoding the TA MUC1 to DCs in lymph nodes to stimulate and increase the population of T lymphocytes that specifically target tumours. The findings of the study indicate that, compared with the use of monoclonal antibodies alone, the concurrent use of vaccines and anti-CTLA-4 monoclonal antibodies in immunotherapy can greatly augment the immune response against tumours.[157]

mRNA Delivery in CAR-Based Cancer Immunotherapy

Chimeric Antigen Receptor (CAR) therapy is a personalized treatment approach involving ex vivo genetic modification of autologous immune cells to selectively target cancer cells.[158] While CAR-T-cell therapy has demonstrated significant efficacy in haematological malignancies, its clinical application to solid tumours is hindered by challenges such as poor tumour penetration, tumour heterogeneity, and immunosuppressive microenvironments.[159,160] mRNA-based CAR therapy offers the advantage of transient CAR expression without genomic integration, which may reduce toxicity and side effects.[161] Additionally, to address the issue of tumour heterogeneity, mRNA NP delivery systems can transport mRNAs encoding Tumor-Specific Antigens (TSAs) or TAAs into the bloodstream via intravenous injection. Upon uptake by APCs, these mRNA NPs facilitate the expression of TSAs/TAAs on the APC membrane. The interaction between these APCs and the corresponding CAR-T cells can effectively promote CAR-T-cell proliferation, enabling them to recognize and destroy targeted tumour cells.[162,163] Therefore, the application of mRNA delivery in CAR-T-cell therapy holds significant promise for advancing cancer treatment (Figure 4).

There have been significant advancements in the application of mRNA delivery in CAR-T-cell therapy. For example, Matthias Stephan et al utilized anti-CD8 antibody-modified NP-like carriers to achieve transient expression of CAR mRNA in circulating T cells in vivo.[164] Repeated administration of these NP formulations induced tumour regression. In a separate study, DLin-MC3-DMA LNPs functionalized with anti-CD5 antibodies were developed as a new platform for delivering anti-fibroblast activation protein (FAP) CAR mRNA, aimed at engineering T cells to target activated fibroblasts.[31] A single intravenous injection of LNPs containing anti-FAP CAR mRNA was able to transiently generate anti-FAP T cells in vivo, ultimately restoring cardiac function and reducing fibrosis in established hypertensive mouse models of cardiac injury. The findings of these studies highlight the potential of mRNA delivery systems in CAR-T-cell therapy for cancer. To address the issue of off-target toxicity caused by the expression of target antigens in normal tissues during CAR T-cell engineering, researchers have established a clinical platform using in vitro transcribed mRNAs encoding CARs (including CD3-ζ and 4–1BB costimulatory domains) to engineer T cells with transient CAR expression. Case reports from two ongoing trials demonstrate that adoptive transfer of mRNA CAR-T cells targeting mesothelin (CAR-T cells) is feasible and safe.[165]

Additionally, several mRNA delivery systems encoding TAAs have advanced to clinical research stages. For example, CV9201, an mRNA drug for non-small cell lung cancer (NSCLC) that encodes five TAAs and is delivered via RNActive technology, demonstrated good tolerability and T-cell responses in 63% of patients in Phase I/II trials. Additionally, in a phase

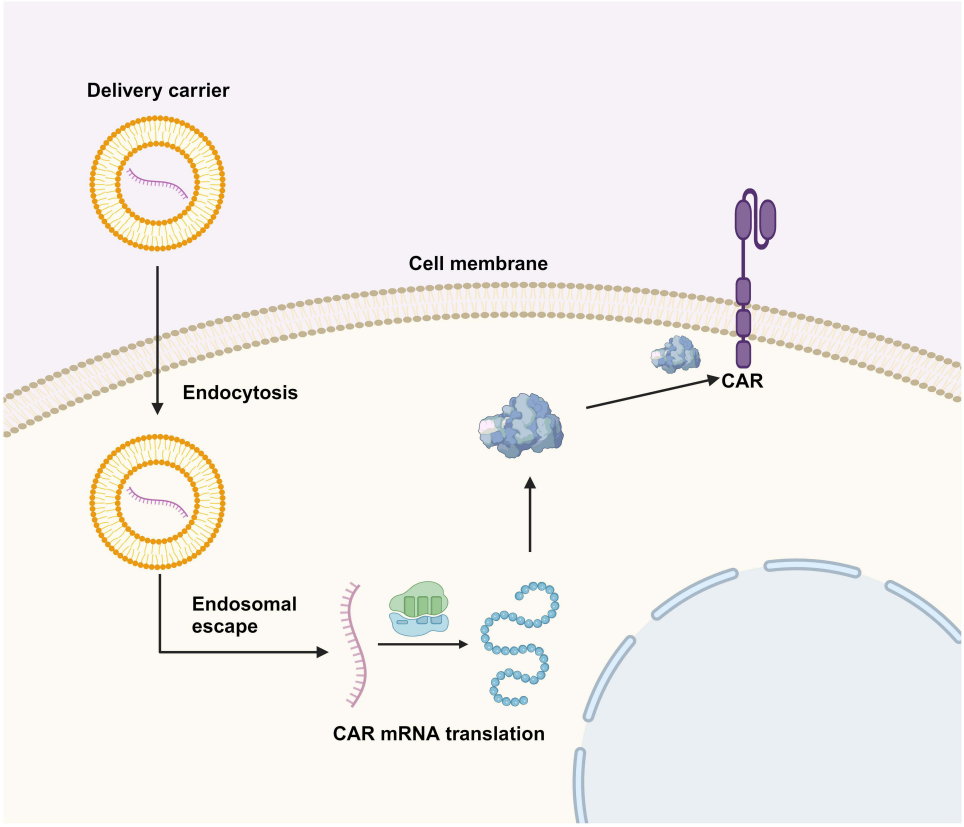

Figure 4 mRNA delivery in chimeric antigen receptor (CAR)-based cancer immunotherapy. Created in BioRender. Dai, C. (2025) https://BioRender.com/p45z401.

Ib trial, combining CV9201 with radiotherapy resulted in 84% of patients showing antigen-specific immune responses.[166,167] Similarly, BNT111, designed for melanoma and encoding four TAAs (tyrosinase, MAGE-A3, NYESO-1, and TPTE), also uses the LPX system. In a Phase I trial, 75% of patients developed an immune response to one or more antigens, and combination therapy with anti-PD-1 antibodies resulted in significant clinical responses. Ongoing Phase II trials are evaluating the combined use of BNT111 and cemiplimab, with preliminary results indicating an increased objective response rate in melanoma patients.[168] BNT211, an mRNA drug encoding the TAA CLDN6, also utilizes the LPX delivery system. Studies have shown that LPX-loaded CLDN6-mRNA effectively induced CAR-T-cell expansion and significantly suppressed ovarian cancer growth in animal models, highlighting its increased therapeutic efficacy.[169] Clinical trials have demonstrated that 57% of patients receiving CLDN6 CAR-T cells achieved partial remission, whereas 14% experienced stable disease, underscoring the potential of this therapy as a treatment option.[170]

Currently, mRNA-CAR-T therapy has its own current situations and advantages. The non-viral vector technique is getting noticed as it can address stability issues by regulating CAR expression with mRNA. Different non-viral delivery systems have unique features, like the flexibility of electroporation and the customizability and low toxicity of nano-carriers. New treatments encoding CAR-related proteins with mRNA also show promise. However, challenges remain. Electroporation may harm cells, and nano-carriers have stability, storage, and biocompatibility issues. Future development can be driven by integrating nanoparticle delivery with gene editing, identifying tumor targets, optimizing delivery carriers, and enabling in vivo CAR-T cell generation.[171]

Owing to their significant cytotoxic potential through their involvement in both innate and adaptive immunity, NK cells are considered promising candidates for CAR therapy alongside T cells.[172] NK-92 is a homogeneous and stable human cell line commonly used as a model for NK cells.[173,174] Given these factors, the NK-92 cell line represents an attractive platform for ready-made CAR therapy, as it also avoids graft-versus-host disease. Indeed, several clinical studies have demonstrated the efficacy of engineered NK-92 cells against various cancers, including haematological

malignancies and solid tumours.[175,176] In one study, researchers used an innovative proprietary delivery platform to produce protein transduction domain (PTD)-IVT-mRNA for expressing CARs on NK-92 cells. CAR-T1E-engineered NK-92 cells were created by incorporating the T1E single-chain variable fragment (scFv) sequence to recognize the ErbB receptor, with either CD28 or 4–1BB as costimulatory signalling domains, and their efficacy was evaluated in two different ErbB (+) cancer cell lines. The results showed that the PTD-IVT-mRNA for the CAR was safely transduced and expressed in NK-92 cells. CAR-T1E-engineered NK-92 cells, which are used as effector cells in coculture assays against HSC-3 (oral squamous cell carcinoma) and MCF-7 (breast metastatic adenocarcinoma) human cell lines, triggered high levels of cell death (25–33%).[177] Studies have shown that the expression of the NKG2D RNA CAR significantly increased the cytotoxic activity of NK cells against various solid tumour cell lines in vitro and provided notable therapeutic benefits for mice with solid tumours. Following the confirmation of the efficacy of NKG2D ligand-targeted mRNA CAR-NK cells in vitro and in animal studies, Lin Xiao et al initiated a pilot clinical trial (NCT03415100), demonstrating that the intraperitoneal infusion and intratumoral injection of CAR-NK cells is a safe and effective local treatment for metastatic colorectal cancer.[178]

Recently, it has been demonstrated that delivering mRNA encoding an anti-GPC3 CAR and an engineered receptor that competitively binds to the phagocytic signal protein CD24 via PPZ-A10 LNPs to target liver macrophages is an effective strategy for increasing the phagocytic activity of CAR-M cells in vivo. In a mouse model of orthotopic HCC, systemic injection of LNPs containing the dual-mRNA formulation resulted in a reduced tumour burden and increased survival time.[179] These promising results underscore the potential of mRNA delivery systems for optimizing CAR-M-cell therapy by enabling precise, adaptable, and safe targeting of TAMs. This approach not only increases the efficacy of CAR-M cells but also represents a significant advancement in the development of personalized cancer treatments, potentially transforming therapeutic strategies for solid tumours and metastatic diseases.

Conclusion and Future Directions

Tumour immunotherapy is a treatment method that attacks tumour cells by activating or regulating the human immune system. The application prospects of mRNA delivery in cancer immunotherapy are extensive. From the initial delivery of naked RNA to the use of liposome carriers, peptide carriers, inorganic NP carriers, and exosomes, mRNA delivery systems have become more stable, more targeted, and exhibit improved release effects. mRNAs have the ability to encode TAAs, immunostimulatory molecules (such as IL-2, IL-12, IL-27, IL-15, IL-23, GM-CSF, IFNγ, etc)., molecules that facilitate the transformation of immune cells with tumor-promoting effects, and molecules that enhance the efficacy of ICIs, as well as those used in CAR-based cell therapy to enhance antitumor immune effects. The combination of mRNA delivery with ICIs can effectively enhance the immune response, thereby overcoming the limitations of single-agent therapies and improving overall efficacy (Figure 5). In CAR-based cell therapy, mRNA delivery technology allows for transient CAR expression, effectively addressing the issue of long-term off-target toxicity associated with traditional CAR-based cell therapies. This strategy not only enhances the targeting ability and efficacy of CAR-T, CAR-NK, and CAR-M cells, but also provides safer and more adaptable treatment options.

However, there are still several issues and challenges associated with tumor immunotherapy based on mRNA delivery. Firstly, the design and functionality of mRNA delivery systems depend on various factors, such as the size, shape, charge, and surface modifications of the carriers. If these parameters cannot be precisely controlled, the carriers may enter off-target cells.[180] To mitigate this risk, researchers are continuously optimizing the design and functionality of delivery systems to enhance their safety and efficacy in vivo. Secondly, there are currently no comprehensive regulations for assessing the safety of mRNA delivery therapeutics. A standardized system for evaluating the efficacy and safety of these therapeutics needs to be established to ensure comparability among different research findings. Thirdly, the stringent storage requirements for mRNA delivery therapeutics and the current inability to achieve large-scale production contribute to high costs for patients.[181] Improving production processes and reducing costs are necessary to make mRNA delivery therapeutics more accessible for widespread use.

In the future, several promising research directions and suggestions can be considered.[182] Firstly, developing novel vectors is of paramount importance. For example, exploring biomimetic vectors that mimic the natural cell communication mechanisms could potentially enhance the delivery efficiency and biocompatibility. Synthetic polymers with unique

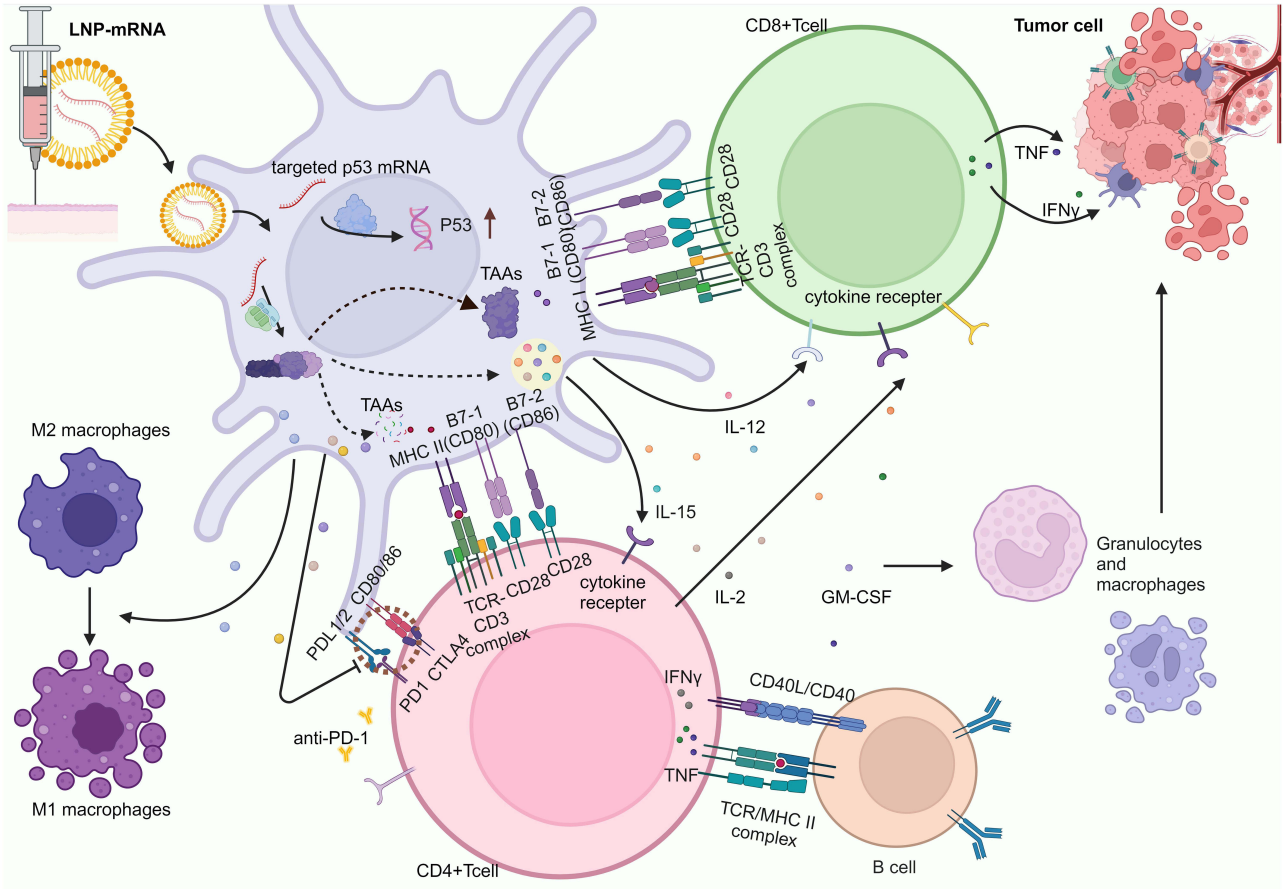

Figure 5 Mechanism of mRNA delivery in tumour immunotherapy. Taking lipid nanoparticle (LNP)-encapsulated mRNAs as an example, these encapsulated mRNAs are introduced into the human body through intravenous injection or other administration methods. They travel via the bloodstream to reach target cells, such as the antigen-presenting cells illustrated in the diagram. Once inside these cells, the LNPs release their mRNA cargo, which then encodes corresponding proteins. These proteins include tumour-associated antigens that activate T cells by binding to signal molecules between antigen-presenting cells and T cells, triggering subsequent antitumour responses. mRNA can also encode immune stimulatory molecules such as interleukins and granulocyte-macrophage colony stimulating factor (GM-CSF), promoting the activation and aggregation of immune cells. Additionally, mRNA can encode signalling molecules that promote the transformation of M2 macrophages into M1 macrophages, thereby improving the anticancer effect. mRNAs that target tumour suppressor genes such as P53 can activate their expression. Furthermore, mRNA delivery therapy can be combined with immune checkpoint blockade therapies or CAR-based cell therapies for cancer treatment, increasing therapeutic efficacy. Created in BioRender. Dai, C. (2025) https://BioRender.com/v27w914.

nanostructures might also be designed to precisely control the release of mRNA and target specific cell types.[183] Secondly, optimizing mRNA sequences is another crucial aspect. By employing advanced bioinformatics tools, researchers can predict and modify mRNA sequences to improve their stability, translation efficiency, and immunogenicity.[184] This could involve minimizing secondary structure formation to facilitate ribosome binding and translation initiation. Furthermore, efforts should be directed towards engineering self-amplifying mRNAs that can produce multiple copies of the encoded protein, thereby amplifying the therapeutic effect. Finally, interdisciplinary collaborations between materials science, bioengineering, and immunology will be essential to address the complex challenges in mRNA-based tumour immunotherapy and drive innovation in this field.[185] It will be necessary to focus on and address these issues to achieve wider application and better development of mRNA-based tumour immunotherapy.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests in this work.

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