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REVIEW

mRNA delivery in cancer immunotherapy

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Abstract Messenger RNA (mRNA) has drawn much attention in the medical field. Through various treatment approaches including protein replacement therapies, gene editing, and cell engineering, mRNA is becoming a potential therapeutic strategy for cancers. However, delivery of mRNA into targeted organs and cells can be challenging due to the unstable nature of its naked form and the low cellular uptake. Therefore, in addition to mRNA modification, efforts have been devoted to developing nanoparticles for mRNA delivery. In this review, we introduce four categories of nanoparticle platform systems: lipid, polymer, lipid-polymer hybrid, and protein/peptide-mediated nanoparticles, together with their roles in facilitating mRNA-based cancer immunotherapies. We also highlight promising treatment regimens and their clinical translation.

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1. Introduction

Two U.S. Food and Drug Administration (FDA)-approved coronavirus disease 2019 (COVID-19) vaccines, BNT162b and mRNA-1273 produced by Pfizer/BioNTech and Moderna, have drawn public attention to the messenger RNA (mRNA)-based therapy[1,2]. Over the last few decades, mRNA has emerged as an innovative and potent therapeutical strategy for cancer treatment[3−6]. Effective mRNA engineering and delivery can be exploited in various biomedical applications through protein replacement, cell engineering[7], gene editing[8,9], and anti-cancer immune activation (Fig. 1)[10−15]. Besides, mRNA therapeutics have shown great advantages in cellular reprogramming by integrating antigen receptors, exhibiting biocompatibility with minimal immunogenicity, and facilitating tissue and organ targeting for local or systemic administration[16−18].

Despite the expeditious development of mRNA therapy, the delivery of the mRNA molecules is facing challenges such as undesired immune responses, exposure to systemic circulations, and inefficient cellular uptake and endosomal escape (Fig. 2)[3]. To encounter these barriers, mRNA is modified to prevent rapid degradation, thereby improving the systemic stability and therapeutic efficiency[19−24]. General modifications include capping 5' guanine and prolonging 3' with repetitive adenosine tails to achieve ribosome attachment for protein translation and transportation to the cytoplasm[20,25]. Transcript stability along with reduced immunogenicity can be achieved by chemically editing ribonucleotides using $N^{1/6}$-methyladenosine, 5-methylcytosine, 5-hydroxymethylcytosine, N^{1}-methylpseudouridine (m1Ψ), or ribose methylation[26,27].

Albeit the accomplishment in optimizing mRNA moieties, targeted cellular uptake is an urgent need for safe, precise, and efficient delivery materials. In recent decades, multiple drug delivery nanomaterials have been proposed and developed, including lipids[28−32], polymers[33], lipid−polymer hybrid[34], and protein/peptide-mediated carriers (Fig. 2)[35,36]. Altogether these materials are advantageous in eluding non-specific binding-induced plasma clearance, penetrating cellular membranes, releasing mRNA drugs through readily endosomal escape, and eliciting adjuvant mRNA therapeutic immune responses[5,7,10,37−40]. In this context, a wide range of mRNA therapeutics are conducted in clinical trials for cancer immunotherapy (Table 1)[20,38,41−45]. This review describes recent advances in four main nanoparticle platforms to deliver mRNAs, including lipid, polymer, lipid-polymer hybrid, protein/peptide-mediated nanoparticles, together with their applications in delivering various mRNA modalities for cancer immunotherapies. We also present future perspectives and research directions for nanoparticle delivery systems and mRNA therapeutics.

2. Nanoparticle-mediated mRNA delivery for cancer treatments

2.1. Lipid nanoparticles (LNPs)

Lipid nanoparticles (LNPs) have emerged as promising and widely used platforms to deliver mRNA therapeutics[45]. Typically, LNPs consist of cationic/ionizable lipids to encapsulate mRNA molecules through electronic interaction, helper lipids such as cholesterol, phospholipid, and polyethylene glycol (PEG)−lipids to maintain particle stability and compatibility. However, certain cationic lipids were reported to induce organ injuries including liver and spleen toxicity[45−47]. In addition, PEG-lipids may induce expression of anti-PEG which might lead to rapid clearance of LNPs[43,45,48−51]. Therefore, efforts have been made to developing lipid materials with high delivery efficiency, organ/cell targeting, and low toxicity. Currently, many mRNA drugs utilizing LNPs as delivery systems are under clinical investigations for the treatment of various solid tumors[20,45,52−54].

In 2018, the FDA approved ionizable lipid, (6*Z*,9*Z*,28*Z*,31*Z*)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate (MC3), for siRNA delivery[55]. More recently, MC3-based LNPs were used as delivery systems in mRNA-based cancer immunotherapy. For example, Wang et al.[56] utilized MC3-based LNPs to deliver mRNAs encoding chemokine (C−C motif) ligand 2 (CCL2) and chemokine (C−C motif) ligand 5 (CCL5) linked by a single domain antibody (BisCCL2/5i). Intravenous administration of the BisCCL2/5i mRNA-LNP blocked both chemokine ligand signaling pathways and polarized the macrophages towards cancer inhibitory phenotype (M1), thereby achieving a 50% survival rate in pancreatic cancer liver metastasis mouse models. Co-administration of BisCCL2/5i mRNA-LNP with PD-1 ligand inhibitor (PD-Li) resulted in a 57% complete response, establishing a potential therapeutic strategy targeting liver malignancy[56].

Also, the need for higher mRNA delivery efficiency drives the optimization of lipid structure[57,58]. For example, Sabnis et al.[59] reported a MC3-derived material lipid 5, heptadecan-9-yl 8-((2-hydroxyethyl) (8-(nonyloxy)-8-oxooctyl) amino) octanoate, by substituting MC3 linoleic tail with a new one containing ester lipids. This optimization exploits a similar cellular uptake mechanism through an apolipoprotein E-mediated low-density lipoprotein-dependent manner with less organelle aggregation driven by spatial configuration[59]. Hewitt et al.[60] leveraged this lipid 5 delivery platform to deliver interleukin 12 (IL12)-encoding mRNA for cancer immunotherapy. The expression of IL12 stimulated the upregulation of T-helper 1 type (TH1) immune response genes, $CD8^{+}$ T cell production, and interferon-γ (IFN-γ) expression, resulting in tumor shrinkage and prolonged survival time. In MC38 mouse models with colon adenocarcinoma, a single intratumorally injection of (murine)IL-12 mRNA-LNP resulted in around 86% complete tumor clearance rate, and nearly all mice were resistant to tumor rechallenge. Furthermore, an (human)IL-12 mRNA-LNP therapy (MEDI1191) is currently active in phase I clinical trial with 87 patients enrolled for solid tumor treatment (NCT03946800)[60]. Hewitt et al.[61] also used lipid 5-based LNP to deliver a tri-mRNA encoding IL-23, IL-36γ, and tumor necrosis factor receptor superfamily member 4 ligand (OX 40L). The IL-23/IL-36γ/OX40L triplet mRNA-LNP is under phase I clinical trial for multiple solid tumor treatment (NCT03739931). In murine colon and hepatocellular carcinoma models, the tri-mRNA-LNP triggered cytokine and chemokine expression and increased complete response rates. Furthermore, four weekly injections of tri-mRNA-LNP resulted in an over 70% complete recovery rate, demonstrating the low toxicity and reproducibility of the lipid 5 delivery system during metabolism and elimination[61]. Likewise, Li et al.[58] delivered mRNA encoding OX40 for expressing costimulatory receptors in combination with an anti-OX40 antibody using a phospholipid-derived LNP system (PL1-LNPs). The combination of PL1-OX40 mRNA and anti-OX40 antibody showed a 60% complete response rate in A20 mouse models. The study also revealed that this combinational therapy significantly enhanced the immune response to anti-programmed cell death protein 1 antibodies (αPD-1) and anti-

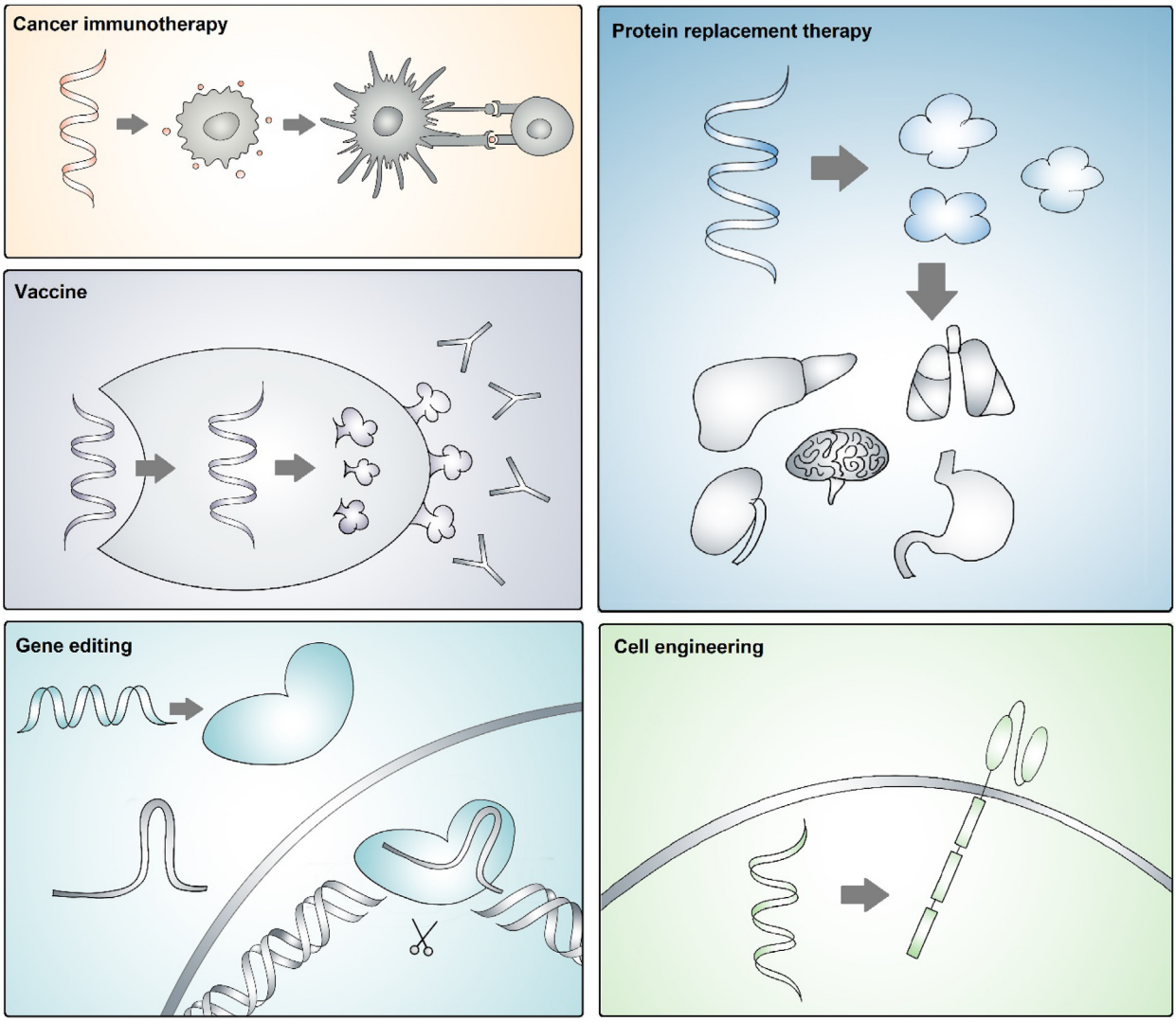

Figure 1 Illustration of representative mRNA-based biomedical applications: cancer immunotherapy by activation and recruitment of immune cells; protein replacement therapy by supplementing defective or absent protein; vaccines by antigen production and presentation; utilization of clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR−Cas9) technologies for gene editing; cell engineering to modify functional cells.

cytotoxic T-lymphocyte-associated antigen 4 antibodies (αCTLA-4) in the B16F10 tumor model[58].

Besides, LNPs are applicable in activating adaptive immune responses by delivering tumor antigens mRNAs to induce long-term anti-tumor effects[62–64]. Recently, Moderna reported the phase I clinical trial results of LNP-delivered mRNA-4157 encoding tumor-associated neoantigens for solid tumor treatment (NCT03897881). Patients were intramuscularly injected once every three weeks for up to 9 cycles. 11 out of 13 patients remained disease free during the study (3 with melanoma, 8 with non-small cell lung cancer (NSCLC), and 2 with colorectal cancer). The trial is currently moving forward to phase II stage for efficacy study with 157 patients enrolled[65]. In a pre-clinical study, Zhang et al.[62] reported that an ovalbumin OVA (257-264)-encoded mRNA-lipid nanoparticle system exhibited therapeutic efficacy in treating OVA-specific mouse colon cancer models. Here, the formulation consists of synthetic lipidoid material C1 (PAMAM dendrimer G0 mixed with 1,2-epoxydodecane) and 1,2-distearoyl-*sn*-glycerol-3-Phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000 (DSPE-PEG2000). The mRNA-lipid nanoparticles enabled the expression of OVA (257-264) in dendritic cells, thereby activating the immune system *via* Toll-like receptor 4 signaling pathway. After three subcutaneous injections in the B16-OVA mouse model, the tumor size in the OVA-mRNA/LNP treated group was 3-fold smaller than that in the PBS control[62]. In another study, Oberli et al.[63] screened an ionizable lipid library and identified a lead formulation using (3,6-bis[4-[bis(2-hydroxydodecyl)amino]butyl]-2,5-piperazinedione (cKK-E12) to delivery mRNAs encoding OVA, glycoprotein 100 (gp100), and tyrosinase-related protein 2 (TRP2). Intravenous injection of OVA mRNA LNP promoted CD8 T cell activation, leading to a two-fold longer survival time and a 2.5-fold reduction in tumor size in the xenografted B16F10 mouse models. The gp100 mRNA treatment dramatically reduced tumor size by more than twofold,

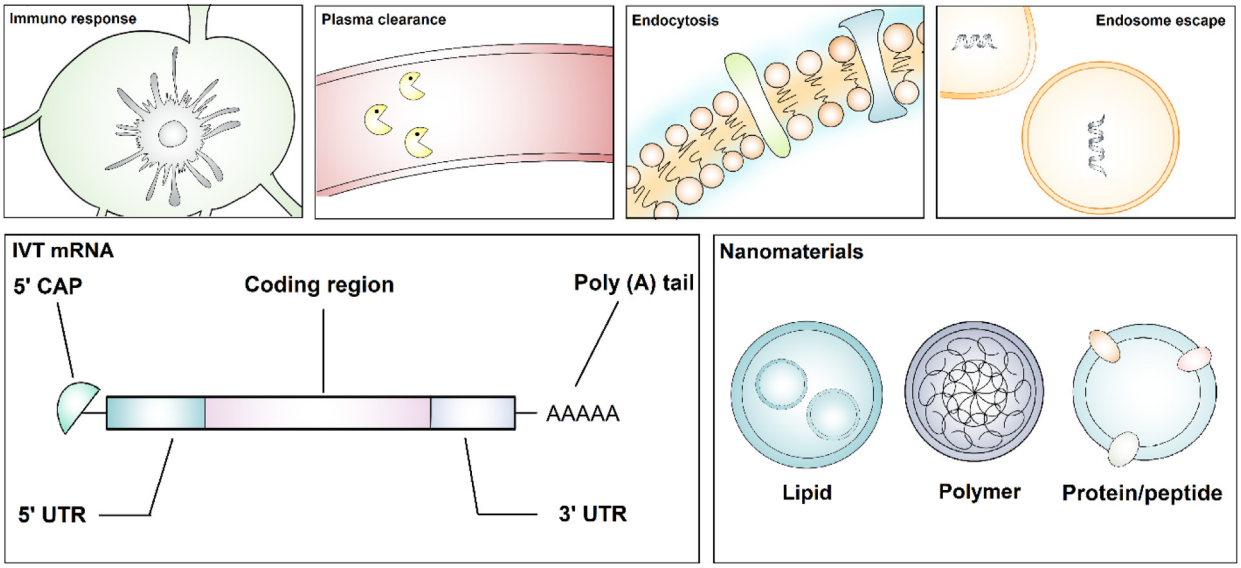

Figure 2 Challenges for mRNA delivery and methods to overcome these barriers, including mRNA modifications and nanomaterial-based delivery platforms.

and co-administration of gp100 and TRP2 mRNAs prolonged the survival time by 20 days[63]. Using the same formulation, Rybakova et al.[64] delivered mRNA encoding anti-human epidermal growth factor receptor 2 (HER2) antibody to xenografted mice with human breast cancer. Compared with HER2-negative tumor growth, HER2-positive tumors were four times smaller after the treatment. Notably, pharmacokinetic results such as the area under the curve and half-life in mRNA therapy outperformed those of single antibody-based administration.

Leveraging LNPs to deliver gene-editing systems is another commonly used strategy for cancer immunotherapy[16,66]. For example, Rosenblum et al.[67] used lipid 8-based LNPs to knock-down polo-like kinase 1 (PLK1) gene in xenograft mouse models by co-delivering Cas9 and single-guide RNA encoding polo-like kinase (sgPLK1). Intracerebral injection of Lipid 8-Cas9/sgPLK1 mRNA into glioblastoma (GBM)-bearing mice resulted in up to 70% gene being edited, and intraperitoneal injection into OV8 xenograft mice achieved 80% gene editing efficiency[67,68]. Liu et al.[69] used the disulfide-integrated LNP BAMEA-O16B to deliver Cas9/sgGFP to human embryonic kidney cells with 90% knockout efficiency. Intravenous injection of BAMEA-O16B-Cas9/sg-proprotein convertase subtilisin/kexin type 9 (PCSK9) demonstrated high accumulation in mice hepatocytes, broadening liver-targeted therapeutic strategies of CRISPR-Cas9 mRNA delivery. Zhang et al.[70] developed a biodegradable lipid-like *N*-methyl-1,3-propanediamine (MPA-Ab) for Cas9/sgGFP delivery and the result showed a 41% reduction in fluorescence signal *via* intratumoral injection into 293T xenografted mouse models. Miller et al.[71] reported that ZA3-Ep10, an LNP composed of a zwitterionic amino phospholipid, cholesterol, and PEG, delivered Cas9/sgLoxP to induce tdTomato expression in livers, kidneys, and lungs of engineered mouse models.

2.2. *Polymeric nanoparticles*

Polymeric nanoparticles have been developed for decades to deliver nucleic acids. The advantages of polymeric nanoparticles lie in their tunability and high encapsulation capability. On one hand, the morphological structures of the polymeric nanoparticles can be tuned by combining different monomer ratios[72,73]. On the other, if positively charged, polymers can bind with mRNAs through electrostatic interactions. Accordingly, polymeric nanoparticles have been formulated for mRNA delivery and applied in cancer immunotherapy. However, particle aggregation issues and low delivery efficiency greatly limit the application of polymeric nanoparticles[43,74]. Therefore, a number of co-polymers have been developed to address these limitations. For example, Zhang et al.[75] developed a polymeric nanoparticle composed of poly (beta-amino ester) (PbAE), poly-glutamic acid (PGA), and Di-mannose moieties to deliver mRNAs encoding transcriptional regulatory factor 5 (IRF5) and inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) to treat ovarian cancer, melanoma, and glioblastoma. Expression and presentation of IRF5/IKKβ in tumor-associated macrophages induced immune responses through boosting secretions of IL-12, IFN-γ, and tumor necrosis factor alpha (TNF-α), resulting in an increase of M1-like macrophage proportion by twentyfolds[75]. To explore a therapeutic efficiency for prostate cancer, the group modified the polymeric nanoparticles to target circulating T cells by replacing the mannose moieties with an anti-CD8 antibody[76]. They transplanted LNCaP C42 prostate cancer cells into NSG mice and treated these mice intravenously with anti-receptor tyrosine kinase-like orphan receptor 1 (antiROR1)-mRNA nanoparticles once per week for six weeks. The survival rate of mice treated with anti-ROR1-mRNA-NP was twofold higher compared with the control group[76].

Polymeric nanoparticles carrying mRNAs encoding specific tumor antigens can also induce long-term effects of antitumor immunity by activating antigen-specific immune cells. Haabeth et al.[77] developed a polymer named charge-altering releasable transporters (CARTs) to deliver an OVA-encoded mRNA in a B-cell lymphoma-bearing mouse model. As synthetic oligomers, CARTs are advantageous in their degradation mechanism from polycationic backbone into neutral molecules, therefore facilitating endosomal escape and mRNA payload release in the cytoplasm. Composed of 13 lipid blocks and 11 cationic blocks, CART D_{13}:A_{11} encapsulated with OVA-mRNA was injected into A20-

Table 1 Recent clinical trials of mRNA-nanoparticle therapeutics against cancer.

Organization	Cancer type	mRNA	Nanoparticle carrier	Administration route	Phase	NCT number
AstraZeneca	Solid tumors	MEDI 1191 (IL-12)	LNP	Intratumoral	I	NCT03946800
BioNTech SE	Prostate cancer	W_pro1/BNT 112 (targeting 5 antigens)	Liposome	IV	I/II	NCT04382898
	Melanoma	BNT 122 (RO7198457)	Lipoplex	IV	II	NCT03815058
		BNT 111	Lipo-MERIT	IV	N/A	NCT02410733
	Colorectal cancer	BNT 122 (RO7198457)	Lipoplex	IV	II	NCT04486378
	Solid tumor	CLDN6	Lipoplex	IV	I/II	NCT04503278
	Head and neck	BNT 113	Lipoplex	IV	II	NCT04534205
	Squamous cell Carcinoma	BNT 113	Lipoplex	ID	I/II	NCT03418480
	Non-small cell lung cancer (NSCLC)	BNT 116	Lipoplex	IV	I	NCT05142189
CureVac AG	NSCLC	mRNA CV9201	RNActive® Protamine	ID	I/II	NCT00923312
		mRNA CV9202	RNActive® Protamine	ID	I/II	NCT03164772
	Hormonal refractory prostate cancer	mRNA CV9301	RNActive® Protamine	ID	I/II	NCT00831467
Merck Sharp & Dohme LLC	Carcinoma, NSCLC, neoplasms	mRNA-5671/V941	LNP	IM	I	NCT03948763
Moderna Therapeutics Inc.	Relapsed/refractory solid tumor malignancies or lymphoma	mRNA-2752 (OX40L, IL-23, IL-36γ)	LNP (lipid5, DSPC, cholesterol, PEG)	Intratumoral	I	NCT03739931
		mRNA-2416 (OX40L)	LNP	Intratumoral	I/II	NCT03323398
	Solid tumors	mRNA-4157 (personalized TAAs)	LNP	IM	I	NCT03313778
	Melanoma	mRNA-4157 (personalized TAAs)	LNP	IV	II	NCT03897881
	Pancreatic, colorectal neoplasms, NSCLC	mRNA-5671/Merck V941	LNP	IM	I	NCT03948763
University Medical Center Groningen	Ovarian cancer	W_ova1 vaccine	Liposome	IV	I	NCT04163094
University of Florida	Melanoma	Autologous total tumor mRNA	DOTAP liposome	IV	I	NCT05264974
	Adult glioblastoma	Autologous total tumor and full-length LAMP mRNA	DOTAP liposome	IV	I	NCT04573140
Changhai Hospital, Stemirna Therapeutics (layout)	Advanced esophageal Squamous carcinoma Gastric adenocarcinoma Pancreatic adenocarcinoma Colorectal adenocarcinoma	Personalized mRNA tumor vaccine	Lipopolyplex	SQ	N/A	NCT03468244

OVA mice. CART D_{13}:A_{11}/OVA-mRNA induced activation of antigen-specific $CD4^+$ and $CD8^+$ T cells, thereby fulfilling the therapeutic efficiency presented by tumor volume reduction and resistance to re-challenge[77,78]. CART delivery system was also used to deliver enhanced green fluorescent protein (EGFP) mRNA to natural killer (NK) cells. Compared to commercially available lipofectamine, CART O_5:N_6:A_9, showed more efficient delivery in NK cells with minimal phenotypic changes[79,80].

Abbasi et al.[81] delivered both Cas mRNA and Ai9 sgRNA to knock out tdTomato STOP cassette using a PEGylated polyplex (polyethylene glycol-*b*-poly{*N*-[*N*-(2-aminoethyl)-2aminoethyl] aspartamide}) (PAsp(DET)). Intraparenchymal injection of PM-Cas/Ai9 sgRNA into transgenic mice models revealed tdTomato expression. Yan et al.[82] formulated a polymeric nanoparticle that contained polyester coupled with A17 (Cysteamine hydrochloride) and C12 (1-Dodecanethiol) modifications. An outer layer of Pluronic F127 was assembled in a ratio of 5% for *in vivo* stabilization by utilizing poly (propylene oxide) segments to leave hydrophobic PEG regions in the outer layer to protect the particle from aggregation and protein absorption. They delivered luciferase-encoded mRNAs to non-obese diabetic/severe combined immunodeficiency mouse models to observe optimal expression *via* fluorescent visualization. The result showed increased signal intensity in the lungs by twofold, indicating the nanoparticle PE4K-A17-0.33C12 a potential platform to deliver mRNAs in lung-targeting cancer treatments[82].

2.3. Lipid-polymer hybrid nanoparticles

Lipid-polymer hybrid nanoparticles generally refer to nanoparticles comprising of polymers and lipid components[83]. Since these hybrid nanoparticles leverage complementary characteristics of lipid and polymer materials, they are considered promising platforms for mRNA delivery. For example, Kong et al.[84] used synthetic lipopolymer hybrid nanoparticles to deliver p53-mRNA for the restoration of tumor suppressors. This formulation consists of an ionizable lipid GO-C14 for mRNA condensation and cytoplasmic transport, a poly-(disulfide amide) that rapidly releases payloads by disrupting disulfide bonds when exposed to a glutathione (GSH)-rich tumor environment, DSPE-PEG2000, and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)] (DMPE-PEG) to prevent degradation. The intravenous delivery of p53-mRNA-NP delayed cancer growth by triggering cell cycle arrest and apoptosis. Furthermore, co-administration of p53-mRNA-NP with the immunosuppressant everolimus achieved almost complete tumor clearance in p53-null liver cancers and metastasis muse models[84]. In addition to p53 restoration for potential cancer therapy, Islam et al.[85] reported the mRNA delivery of PTEN, another tumor suppressor gene, for prostate cancer therapy. Modifications to the previous formulation include replacing poly (disulfide amide) (PDSA) with poly (lactic-*co*-glycolic acid) (PLGA) for core stabilization with higher biocompatibility and biodegradability, as well as substituting SPE-PEG2000/DMPE-PEG mixture with ceramide-PEG for higher drug retention. Facilitated with micropinocytosis and released by the proton-sponge effect, PTEN-mRNA-PGCP NP reduced PTEN-null PCa LNCaP and LN3 subclone cell viability *via* inhibiting PI3K-AKT signaling pathway. Six intravenous injections of PTEN-mRNA-NP in PCa xenografts and advanced PCa mouse models showed tumor sizes five and fourfold smaller than those treated with PBS and EGFP-mRNA-NP controls, respectively[85]. In both studies, restoration of tumor suppressor genes in null animal models provides a potential approach to reactivate cancer immune responses to kill tumors.

Lipid-polymer hybrid nanoparticles can also deliver tumor-specific antigens to activate adaptive immune responses to fight against cancers. For example, Persano et al.[86] developed a lipopolyplex (LPP) to deliver OVA-encoded mRNA to treat lung metastases of murine B16 tumors. This hybrid nanoparticle contains a poly(beta-aminoester) core wrapped in a lipid shell made of 49% 1,2-dioleoyl-*sn*-glycero-3-ethylphosphorylcholine (EDOPC), 49% DOPE, and 2% DSPE-PEG-2000. Following cellular entry *via* micropinocytosis, OVA-mRNA-LPP promoted dendritic cell maturation indicated by upregulated expression of INF-α, INF-β, and IL-12 in C57BL/6 mice compared with PBS control. In B16-OVA lung metastasis mouse models, the reduction in the number of lung nodules, overexpression of IFN-γ, and activation of TRP2-positive $CD8^+$ T cells demonstrated anti-tumor efficiency after three OVA-mRNA-LPP tail vein administrations[86].

The introduction of anti-angiogenic factors by hybrid nanoparticles is another approach to inhibit tumor growth by minimizing the tumor vascular network and preventing newly formed blood vessels. Uchida et al.[87] designed a lipopolymer-based nanoparticle to deliver anti-angiogenic protein sFlt-1-encoded mRNA to BxPC3 xenograft mouse models. The nanoparticles were prepared by introducing a cholesterol moiety to the block polymer made of poly[*N*-{*N*''-[*N*'''-(2-aminoethyl)-2-aminoethyl]-2-aminoethyl}2-aminoethyl) aspartamide] (PEG-PASp(TEP)-Chol). Compared with HEPES-treated controls, intravenous administration of the sFlt-1 mRNA-loaded lipopolymer NPs substantially decreased the relative tumor volume by fourfold, along with reduced blood vessel density around the tumor area[87].

Zhao et al.[88] reported luciferase mRNA delivery *via* a lipid polymer hybrid nanoparticle (LPN) composed of a PLGA4 core and a lipid shell prepared from *N*1,*N*3,*N*5-*tris*(3-(didodecylamino) propyl)benzo-1), 3,5-tricarboxamide (TT3), DOPE, cholesterol, and DMG-PEG2000. Luci-mRNA/PLGA4-LPN increased luciferase intensity by twofold compared with TT3 lipid nanoparticles. Xiong et al.[89] formulated a theranostic dendrimer-based lipid nanoparticle (DLNP) from dendrimer 4A3-SC8, DOPE, Cholesterol, and PBD-lipid which was prepared by a PEG-DMG-2000 connected to 4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacene (BODIPY) core with an aryl linker. High bioluminescence intensity around tumor areas was observed in SUM159 breast cancer xenografted mouse models 6 h post intratumoral injections of Luci-mRNA-DLNP.

2.4. Protein/peptide-mediated nanoparticles

Proteins or peptides-mediated nanoparticles are another important mRNA carrier. Due to the nature of macro-biomolecules, the sizes of protein/peptide-mediated nanoparticles are large enough to avoid rapid clearance in the blood circulation before reaching target sites. Moreover, protein/peptide employs a unique mechanism to penetrate targeted cell membranes through direct translocation *via* ligand-receptor interactions[35,36,90].

Clinical studies have been conducted for cancer immunotherapy by delivering mRNAs encoding tumor-associated antigens through a self-adjuvant RNActive® platform, a mixture of protamine-mRNA and free mRNA at a weight ratio of 1:2. For example, CV9103 (NCT00831467) is a clinical trial that investigates the use of RNActive®/mRNAs encoding PSA, PSCA, PSMA, and STEAP1 for prostate cancer treatment[91]. CV9201 (NCT00923312) delivers MAGE-C1, MAGE-C2, NY-ESO-1, survivin, and 5T4-encoded mRNAs to treat stage IIIB/IV disease of NSCLC[92,93]. Patients in both studies were administered intradermally in weeks 1, 3, 7, 15, and 23. In the CV9301 study, 76 percent of patients elicited detectable cellular immune responses. Among these responders, the median overall survival was 29.3 months for non-metastatic disease patients and 31.4 months for metastatic disease patients[91]. In CV9201 phase II trial, the survival rates for 1, 2, and 3 years were 44.4%, 26.7%, and 20.7%, respectively[93].

Besides, pre-clinical studies involved delivering OVA, a commonly used tumor antigen, in the form of mRNA by Udhayakumar et al.[94] using cell-penetrating peptide nanocomplexes. The nanoparticle contained a RALA peptide made of 30 amino acids with rich arginine and lysine to condensate mRNA by charge and an alanine- and leucine-rich hydrophobic layer shield. The OVA-mRNA/RALA complex increased inductions of INF-β and IL-6 transcript levels by three-hundred and seven fold, respectively. Remarkably, the RALA complex, if incorporated with pseudouridine and 5-methylcytidine modified mRNA, instigated cytotoxic T-cell immunity to achieve a 70% antigen-specific killing rate[94].

Mai et al.[95] delivered mRNA encoding cytokeratin 19 (CK19), a protein widely expressed in epithelial cell membranes, to elicit anti-tumor responses through a protamine-liposome nanoparticle system containing protamine, dioleoyl-3-trimethylammonium propane (DOTAP), cholesterol, and DSPE-PEG. The cell-penetrating properties of these arginine-rich peptides allowed

CK19-mRNA-NP to target aggressive Lewis lung cancer cells in a mouse model. Through intranasal administration, CK19-mRNA-NP accelerated dendritic cell activation by overexpressing MHC II molecules and CD86, resulting in substantial inhibitory effect by at least four-fold reduction of tumor volume[95]. A similar protamine-liposome system was applied to deliver interleukin-encoded mRNAs for the regulation of cell growth, differentiation, and activation, thereby modulating inflammation and immune responses. Lei et al.[96] delivered IL15-mRNA to fight against colon cancer *via* a protamine/liposome system (CLPP) incorporating 50% protamine, 25% DOTAP, and 25% cholesterol. The overexpression of IL-15 proteins by IL15-mRNA-CLPP subsequently increased lymphocyte viability, activated T cells and NK cells, and initiated TNF-α signaling pathway. The safety and efficiency of CLPP were also demonstrated in C26 xenograft and C26 lung metastasis mouse models through intraperitoneal administration, which was evidenced by a four-fold reduction in tumor weight without significant pathological changes[96].

The CLPP carrier system was also utilized to deliver an mRNA encoding Survivin-T34A to treat C26 colon cancer[97]. Through a lipid raft-mediated pathway, Survivin-T34A-mRNA-CLPP penetrated cell membranes and exposed mRNAs for protein expression. The result showed a 56% cellular proliferation inhibition supported by elevated expression levels of TNF-α, IFN-γ, and IL-6 cytokines, along with the infiltration of $CD4^+$, $CD8^+$, NK, and macrophage cells, shifting the cellular microenvironment to the tumor-suppressive phase. In C26 abdominal cavity and pulmonary metastatic mouse models, therapeutic efficiency achieved a thirty fold reduction in nodule weight and 78% inhibition of metastatic growth[97]. Wang et al.[98] delivered herpes simplex virus 1-thymidine kinase (HSV1-tk)-encoded mRNA to nude mice bearing NSCLC H460 xenografts. The lipid/protamine/mRNA (LPR) formulation contains a protamine-mRNA core encapsulated by a self-assembling DOTAP/cholesterol liposome, surrounded by DSPE-PEG and DSPE-PEG-AA. The coadministration of ganciclovir (GCV) as a prodrug system inhibited tumor growth and reduced tumor volume by more than twenty fold compared with the untreated group[98].

Gao et al.[99] designed a fusion of two cell-penetrating peptides, cRGD-R9, to facilitate cellular uptake by a cyclic Arg-Gly-Asp for delivering mRNAs encoding Bim, another suicide gene, into C26 colon xenografted mice. By fusing these peptides to DMP nano skeletons containing DOTAP and methoxy poly (ethylene glycol)-poly(ε-caprolactone) (mPEG-PCL), they described multiple cell-penetrating mechanisms of the formulation, including pinocytosis, caveolin- and clathrin-mediated endocytosis. Therapeutic data showed a more than 97% inhibition of C26 cancer cell growth and tumor shrinkage in pulmonary metastasis mouse models through local administration[99].

3. Conclusions

The nanoparticle advancements have led to an increasing number of mRNA-related clinical trials over the past decade, particularly after the emergence of COVID-19 mRNA vaccines. Despite the significant progress, mRNA-based cancer immunotherapy is still in its early stage and thus more investigations are needed to elaborate pharmacokinetic profiles, improve the efficacy, and minimize toxicity[43,90,100]. Although utilizations of highly biodegradable materials greatly improve the delivery of mRNA-based drugs, how to maintain the balance between efficacy and safety remains a concern for clinical translation of mRNA-based cancer treatment. Collectively, with the development of functional nanoparticle-based delivery systems, mRNA may provide revolutionary and promising therapeutic effects for cancer immunotherapy.

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Author contributions

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Conflicts of interest

Yizhou Dong is a scientific advisory board member of Oncorus Inc, Arbor Biotechnologies, and FL85. Other authors declare no conflicts of interest.

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