Acta Pharmaceutica Sinica B 2025;15(1):52–96

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

# Advances and prospects of RNA delivery nanoplatforms for cancer therapy

**Mohamed S. Attia, Gregor Kijanka, Nam-Trung Nguyen, Jun Zhang, Hongjie An***

*Queensland Micro and Nanotechnology Centre, Griffith University, Nathan, QLD 4111, Australia*

Received 25 June 2024; received in revised form 17 July 2024; accepted 10 August 2024

**KEY WORDS**

Cancer therapy;
mRNA vaccine;
RNA interference;
Nanoplatforms;
Gene delivery;
Immunotherapies;
Gene silencing;
Neoantigens

**Abstract** Modern oncology is rapidly evolving, driven by recent advances in RNA-based therapeutics. As new emerging cutting-edge technology, mRNA vaccines hold excellent promise for encoding immunostimulatory molecules, tumor-associated antigens, neoantigens, and chimeric antigen receptors for T-cell reprogramming. RNA interference tools enable highly effective post-transcriptional gene silencing that has rapidly progressed towards more tailored antitumor treatments targeting key molecular players in tumor progression and drug resistance. The inherent challenges and limitations of RNA-based tools, such as size, low stability and surface charges hindering direct cell entry, along with the short circulatory half-life and rapid clearance, call for new and improved RNA delivery systems enabling enhanced gene delivery. Nanoplatforms, particularly certain types of lipid, polymeric nanoparticles and inorganic nanoparticles, provide designed means to address the challenges of RNA delivery and cellular uptake. This paper explores the challenges and barriers while giving insight into the future perspective of RNA-based cancer therapeutics in the context of delivery nanoplatforms and the challenges during development.

© 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

*Corresponding author.
E-mail address: hongjie.an@griffith.edu.au (Hongjie An).

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.09.009

2211-3835 © 2025 The Authors. Published by Elsevier B.V. on behalf of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## 1. Introduction

The incidence of malignancies has grown steadily over the past few decades, contributing to significant mortality worldwide. Despite advancements in interventions like tumor resection, radiotherapy, and chemotherapy, cancer continues to claim many lives. Even though conventional cancer treatments primarily target the main malignant lesion, effectively curtailing metastatic dissemination to other organs remains a hurdle to successful treatment[1]. Ongoing efforts aim to eradicate these circulating and metastatic tumor cells, among which innovative RNA-based therapeutics such as immunotherapeutic messenger RNA (mRNA) vaccines and antisense therapy are emerging promising avenues in this regard[2,3].

The first acknowledgement of the active role of the immune system can be traced back to around three millennia ago when the ancient Egyptians observed tumors vanishing following infections[4]. In 1868, Busch noted tumor regression in erysipelas-infected cancer patients[5], and the hypothesis of bacterial infections for cancer treatment was further confirmed and advanced by William Coley[6,7]. Morales et al.[8], in 1976, further implemented Coley's concepts by applying Bacillus Calmette-Guérin (BCG) vaccine to treat bladder cancer. In 1909, Paul Ehrlich laid the foundation of the immunosurveillance hypothesis, describing the immune system as a critical defense mechanism to thwart the development of carcinomas by suppression and elimination of nascent cancer cells[9,10]. Nowadays, immunotherapies are poised as key inspiring frontiers for the future of oncology[11], to restrict tumor growth and extend patient survival through the enhancement of the patient's antitumor immunity while surmounting the suppressive tumor microenvironment (TME)[12].

Immunosuppressive patients are facing a higher risk of developing cancers[13,14], frequently seen in transplant patients, which highlights a strong link between immunity and cancer. Cancer immunotherapies deploy the immune system as a tumor cell-killing tool, despite the risk of triggering undesirable immune-related adverse events (irAEs). Modern immunotherapies have shown the ability to induce long-term remission with fewer side effects than standard chemotherapies[15]. Antibody-, protein-, and cell-based approaches have now been established as successful immunotherapies, with mRNA-based therapies emerging as an advancement of protein replacement strategies addressing some of the constraints associated with existing treatments[16].

The journey of mRNA therapies in medical research arose in the late 1970s with liposomal *in vitro* delivery of globulin-encoding mRNA into human epithelial carcinoma cells (Fig. 1)[17]. Breakthroughs were made with the laboratory synthesis of biologically active mRNA *via* a bacteriophage-derived enzyme, SP6 RNA polymerase, in 1984[18]. Later in 1993, Transgène developed a liposomal formulation for mRNA that elicited an antiviral immune response in mice[19]. Vical and Merck attempted to develop an mRNA vaccine for influenza in 1991; however, the efforts were aborted due to the high manufacturing costs of mRNA[20]. Although this era predominantly favored DNA vaccines, efforts to scale-up mRNA production laid the basis for future mRNA vaccine research.

Clinical trials demonstrated the promise of mRNA-based vaccines when introduced through safe and efficient transfection methods. Many ongoing preclinical and clinical developments explore mRNA-based vaccines and RNA interference technologies, offering a personalized approach to cancer treatment that leverages the body's immune response[15]. *In vitro* transcribed mRNAs as cancer vaccines present precision and versatility in controlled protein expression without the need for nuclear entry[21]. Direct intramuscular injection of mRNA-encoding viral, tumor-associated antigens and neoantigens has been demonstrated to express vaccine components effectively. In addition, *ex vivo* transfection of dendritic cells (DCs) with mRNA and reintroduction of mRNA DC as a vaccine presents an alternative approach to physical methods of vaccine delivery[22]. The recent development of COVID-19 vaccine technology during the pandemic has sparked interest in using this technology for cancer treatment. Despite the pre-established efficacy demonstrated by mRNA vaccines, their potential as cancer treatments is still under evaluation, with ongoing clinical trials for safety and efficacy assessment.

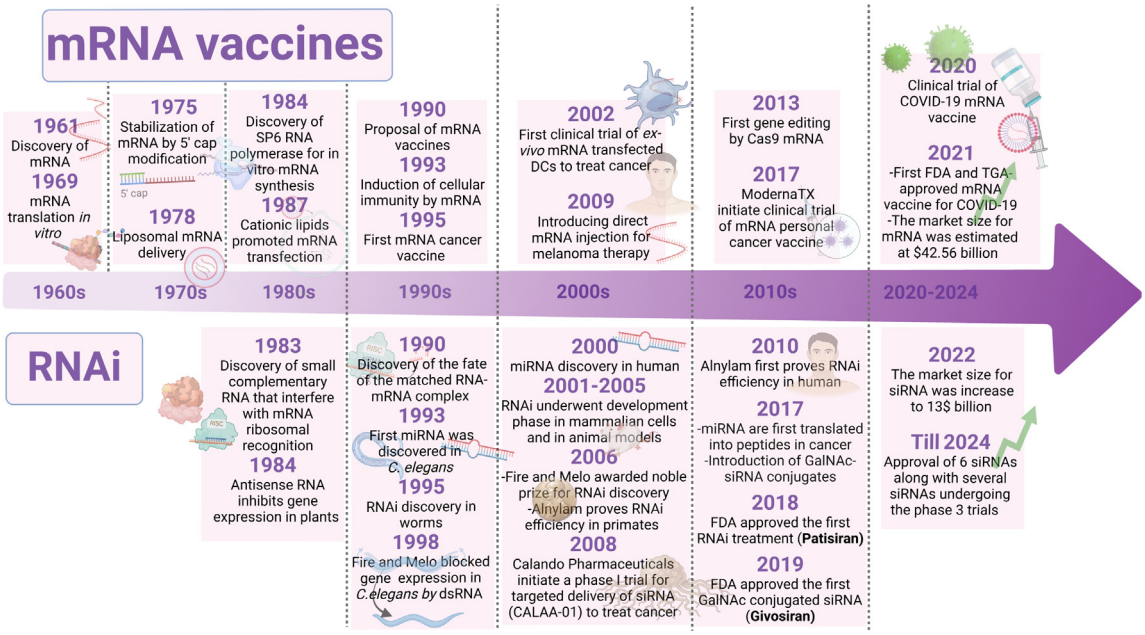


**Figure 1** Timeline for the progress of mRNA and RNAi therapeutics. Figure was made by Biorender.com.

Since 1998, researchers have also exploited RNAi tools that comprise small interfering RNA (siRNA), circular RNA, short hairpin RNA (shRNA), and microRNA (miRNA) to identify cancer-related genes, elucidate complex regulatory pathways and validate potential therapeutic targets[23]. RNAi has paved the way for tailored cancer treatments by targeting key drivers of cancer progression and drug resistance, which in turn inhibit tumor cell proliferation and are promising in treating cancer. Recent advances in the delivery of nucleic acids using lipoplexes, polyplexes, lipoplexes, or exosomes have overcome some of the challenges related to instability, innate immune responses, and ineffective transfection[24].

This paper reviews the new development of RNA therapeutics in cancer therapy along with the recent nanotechnological advances in RNA delivery. In addition to discussing the challenges and struggles impeding their efficacy, the article also presents a glimpse into these therapies' promise and prospects for cancer treatment.

## 2. The role of RNA therapeutics in cancer therapy

Recent reports demonstrated the potential of a wide variety of RNA-based therapies, with some approved by the US Food and Drug Administration (FDA). These include coding mRNAs and noncoding RNAs such as antisense oligonucleotides (ASOs), siRNAs, miRNAs, and RNA aptamers[25]. Recently, mRNA therapeutics have been employed for protein replacement therapy, generating antigens that trigger immunity or expressing components of gene-editing tools (Fig. 2)[26].

The development of RNA-based therapeutics emphasizes two key strategies. The first relies on mRNA vaccines that encode specific antigens or defective proteins/peptides to transiently initiate targeted immune responses. Presenting cancer antigens during immune stimulation, mRNA vaccines can induce tumor-specific T-cell responses, ultimately leading to tumor apoptosis. mRNA vaccines can potentially deliver tumor-specific antigens (TSA) or tumor-associated antigens (TAAs), along with activating innate immunity. Second, RNAi are designed to attach to the complementary sequences in endogenous RNA transcripts and alter their processing[27]. The RNAi approach enables precise regulation of intracellular checkpoints, offering druggable targets for precisely controlling inflammatory and immune reactions. Table 1 presents the clinical translation of mRNA-based vaccines and RNAi approach, accompanied by details of the administration route, dosing, delivery vector, or method for each registered trial, along with the corresponding treated cancer type.

### 2.1. *mRNA vaccines*

Common vaccine strategies that include attenuated or inactivated viral vaccines have proven successful in defense against the progression of infections. However, this approach is not applicable to the evolution of tumor vaccines[28]. A significant step forward has been made in cancer immunotherapy and infectious disease prevention with mRNA-based therapies. This has been seen by investigational vaccines for cancer, as well as influenza, HIV, and Zika viruses, and ultimately, the approved COVID-19 vaccines[29,30]. A notable advantage over traditional vaccines is that mRNA development is fast and scalable, coupling with mRNA's dual activity: activating immune system response while translating immunostimulant antigens or molecules[28,31]. Moreover, mRNA bears a low mutation risk since nuclear entry and transcription are

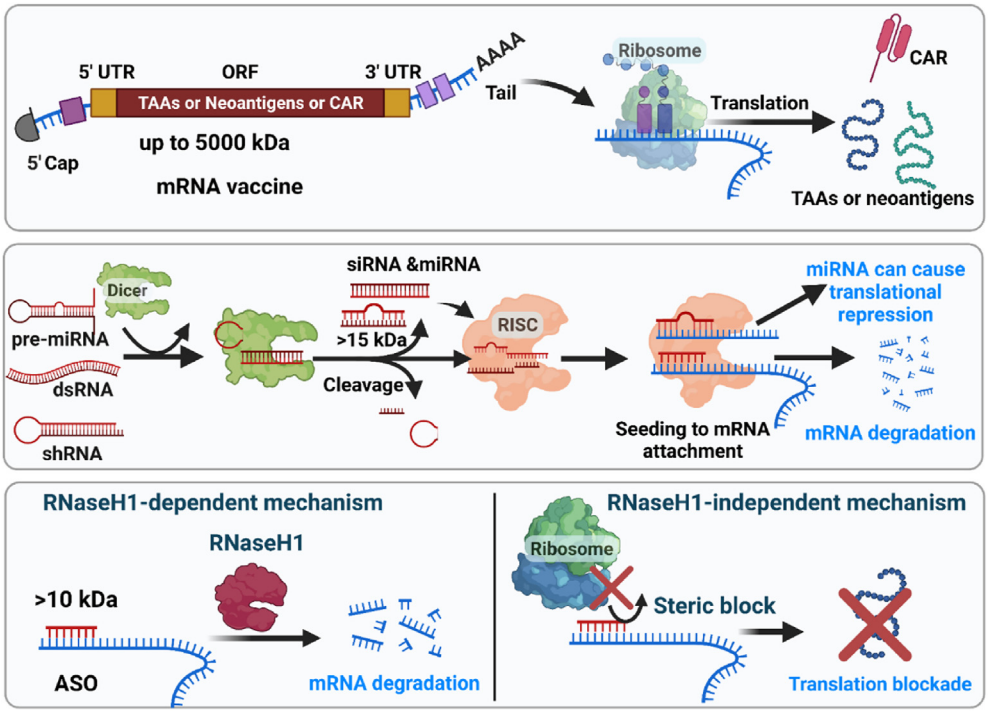


**Figure 2** Mechanism of RNA therapeutics for cancer immunotherapy. mRNA vaccines function through transcription into antigens or specific immunostimulatory proteins. Similarly, siRNAs and miRNAs interfere with mRNA, causing degradation or translational repression. ASOs act through RNaseH1-dependent and independent mechanisms, resulting in the blocking of translation or mRNA degradation. Figure was made by Biorender.com.

**Table 1** Completed and ongoing clinical trials using mRNA and RNAi-based therapeutics for cancer therapy.

| Cancer type | Target protein expression/knockdown | Product name | Delivery system/ method | Route of administration | Dosing | Phase | Trial start date | Completion date | National Clinical Trial (NCT) Identifier Numbers |
|---|---|---|---|---|---|---|---|---|---|
| | mRNA | | | | | | | | |
| MPM | Anti-mesothelin | Mesothelin redirected T-cells | Electroporation | I.V. | Participants received one dose of $1 \times 10^8$ cells on Day 0, followed by one dose of $1 \times 10^9$ CAR-T cells on Day 7<br>Participants received three doses of $1 \times 10^8$ cells on Days 0, 2, and 4, followed by three doses of $1 \times 10^9$ T-cells on Days 7, 9, and 11 | I | 2011 | 2015 | NCT01355965 |
| Metastatic PC | | | Electroporation | I.V. | 1 to $3 \times 10^8$ T-cells/$m^2$ were given thrice weekly for three weeks | I | 2013 | 2017 | NCT01897415 |
| Metastatic BC | Anti-c-Met | cMet redirected T-cells | Electroporation | I.T. | Participants received a single dose of two levels, $3 \times 10^7$ or $3 \times 10^8$ cells | 0 | 2013 | 2018 | NCT01837602 |
| B-CLL, and NHL | Anti-CD16V-BB-ζ | CD16V-BB-ζ redirected T-cells | Electroporation | I.V. | In phase 1a: one dose of T-cells ($0.5 \times 10^6$/kg) was administered two days after rituximab (500 mg/$m^2$). If there is no toxicity and a positive response, a second dose ($0.5 \times 10^7$/kg) may be given 28 days later. IL-2 (1 million IU/$m^2$/day) was given subcutaneously for three days post-infusion<br>In phase 1b: 4 infusions of T-cells ($0.5 \times 10^7$/kg) at 10-day intervals, each after rituximab (375 mg/$m^2$). IL-2 was administered only after the first two infusions | I/II | 2014 | Unknown | NCT02315118 |
| Solid tumors | Neoantigen | mRNA-4157 (V940) | LNP | I.M. | Participants receive mRNA-4157 *via* | I | 2017 | Ongoing | NCT03313778 |
| Stage III/IV melanoma | Neoantigen | mRNA-4157 (V940) | LNP | I.M. | I.M. every 21 days for up to 9 cycles | IIb | 2019 | 2023 | NCT03897881 |
| Locally advanced tumors (melanoma, NSCLC, BC, CC, TNBC, RC, and HNC) | Neoantigen | Cevumeran or RO7198457 | Lipoplex | I.V. | RO7198457 was tested at 25, 38, 50, 75, and 100 μg. Doses of 25, 38, and 50 μg were combined with 1200 mg atezolizumab every three weeks. In the induction phase, RO7198457 was given weekly and biweekly for eight doses, with a boost in cycle seven and maintenance every eight cycles. Atezolizumab was administered on Day 1 of each 21-day cycle | I | 2017 | Ongoing | NCT03289962 |
| PC | | | | | The dose amount was not stated. Administration of 8 weekly doses of Cevumeran starts at week $9 \pm 2$ post-tumor resection | I | 2019 | Ongoing | NCT04161755 |

(*continued on next page*)

**Table 1** (*continued*)

| Cancer type | Target protein expression/knockdown | Product name | Delivery system/ method | Route of administration | Dosing | Phase | Trial start date | Completion date | National Clinical Trial (NCT) Identifier Numbers |
|---|---|---|---|---|---|---|---|---|---|
| Refractory LC | HBsAg | HBV mRNA vaccine | – | I.M. | Starting at 20 μg, doses increased per scheme. Each subject received one dose weekly for four weeks, with a 5th dose after one month | I | 2023 | Ongoing | NCT05738447 |
| Refractory advanced solid tumors | CLDN6 | BNT211 | Lipoplex | I.V. | Patients received a single intravenous infusion of T-cells at a dose of $1 \times 10^7$ or $1 \times 10^8$ cells following lymphodepletion with cyclophosphamide (500 mg/m$^2$) and fludarabine (30 mg/m$^2$) on Days −5 to −3. CARVac is administered at 25 μg, with subsequent doses at 50 μg every 3 or 6 weeks | I/IIa | 2020 | Ongoing | NCT04503278 |
| T-ALL and LBL | CD7 | WU-CART-007 | – | I.V. | Patients are administered four dose levels (ranging from 1 to $9 \times 10^8$ cells) of a single infusion of WU-CART-007 on Day 1, following lymphodepletion with fludarabine and cyclophosphamide from Days −5 to −3 | I/II | 2022 | Ongoing | NCT04984356 |
| Advanced solid tumor | siRNA Protein kinase N3 | Atu027 | Liposome | I.V. | Single and repeated infusions of Atu027 twice weekly over a 28-day cycle, with doses escalating from 0.001 to 0.336 mg/kg, were well tolerated, as was the maximum dose | I | 2009 | 2012 | NCT00938574 |
| Solid tumors | VEGF and KSP | ALN-VSP02 | LNP | I.V. | Patients were allocated to 7 dose levels (0.1−1.5 mg/kg) | I | 2010 | 2012 | NCT01158079 |
| AAC and NETs | PLK1 | TKM-080301 | LNP | I.V. | Patients administered 0.6 or 0.75 mg/kg per week for up to 18 cycles | I/II | 2010 | 2015 | NCT01262235 |
| Primary or secondary LC | PLK1 | TKM-080301 | | H.I.A. | The starting dose received was 4 mg/m$^2$, given every two weeks for up to 12 doses | I | 2011 | 2012 | NCT01437007 |
| Advanced HC | PLK1 | TKM-080301 | | I.V. | The starting dose was 0.3 mg/kg, increasing to 0.75 mg/kg, with a recommended MTD of 0.6 mg/kg. It was administered once weekly for three weeks and repeated every 28 days for up to 6 cycles | I/II | 2014 | 2016 | NCT02191878 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Advanced PAAC | KRAS | siG12D LODER™ | Biodegradable polymeric implant | I.T. | In three escalating cohorts, a single dose of siG12D-LODERs (0.025, 0.75 and 3.0 mg) was administered, followed by weekly gemcitabine | I/IIa | 2010 | 2013 | NCT01188785 |
| PDAC and PC | KRAS | siG12D LODER™ | | I.T. | 2.8 mg of siG12D-LODERs every 12 weeks alongside chemotherapy | II | 2012 | Unknown | NCT01676259 |
| Advanced solid tumors | EphA2 | EPHARNA | Liposome | I.V. | NS (dose-escalation study) | I | 2012 | 2025 | NCT01591356 |
| PAAC | Protein kinase N3 | Atu027 | Liposome | I.V. | Atu027 was given at 0.253 mg/kg, and gemcitabine at 1000 mg/m$^2$ was administered on Days 1, 8, and 15, followed by a one-week off | Ib/IIa | 2013 | 2016 | NCT01808638 |
| GSM and recurrent GBM | BCL2L12 | NU-0129 | Spherical Nucleic Acid Gold Nanoparticle | I.V. | Patients received 1, 4, and 8 mg/kg of siRNA doses | 0 | 2017 | 2022 | NCT03020017 |
| Metastatic PAAC, PDAC, and stage IV PC | KrasG12D mutation | KRAS G12D siRNA | MSCs-derived exosomes | I.V. | NS, dose-escalation study. Doses are given on Days 1, 4, and 10, with cycles repeating every 14 days for up to 3 cycles | I | 2018 | Ongoing | NCT03608631 |
| | miRNA | | | | | | | | |
| MPM, and NSCLC | Mir 16 | TargomiRs miRNA mimic | Nonliving bacterial nanocells (TargomiRs) | I.V. | All patients began on a microdose of 1 billion once a week and escalated to the full phase 1 dose on week 3 | I | 2015 | 2017 | NCT02369198 |
| Lymphomas and leukemia | Mir 155 | MRG 106 (Cobomarsen) Anti-miR | Chemical modification (LNA) | I.T. I.V. | Patients received up to five I.T. injections of Cobomarsen over 15 days, starting at a maximum dose of 75 mg, with possible dose reductions to find the minimum effective dose. Otherwise, they received S.C. Cobomarsen (900 mg) on Days 1, 3, and 5, then weekly until intolerance, significant side effects, or disease progression | I | 2015 | 2020 | NCT02580552 |

AAC: adrenal cortical carcinoma; BC: bladder cancer; BC: breast cancer; B-CLL: B-cell chronic lymphocytic leukemia; CC: colorectal cancer; GSM: gliosarcoma; GBM: glioblastoma; HC: hepatocellular carcinoma; H.I.A.: hepatic intra-arterial; HNC: head and neck cancer; I.M.: intramuscular; I.T.: intratumoral; I.V.: intravenous; LC: liver cancer; LNA: locked nucleic acid; LBL: lymphoblastic lymphoma; MPM: malignant pleural mesothelioma; MSCs: mesenchymal stromal cells; MTD: maximum tolerated dose; NA: not applicable; NETs: neuroendocrine tumor; NHL: non-Hodgkin's lymphoma; NS: not stated; NSCLC: non-small cell lung cancer; PAAC: pancreatic adenocarcinoma; PC: pancreatic cancer; PDAC: pancreatic ductal adenocarcinoma; RC: renal cancer; S.C.: subcutaneous; TNBC: triple negative breast cancer; T-ALL: T-cell acute lymphoblastic leukemia.

not necessary for translation into amino acids, and mRNA is rapidly eliminated by cellular enzymes.

The large size of mRNA molecules and their high negative charges make them inaccessible to the body and must be delivered with reliance on delivery systems[32]. When handling mRNA, a significant concern is the maintenance of its stability[33], whether in clinical or preclinical studies. mRNA is highly susceptible to oxidative damage and enzyme degradation, both *in vitro* and *in vivo*, and therefore, must be handled under sterile and RNase-free conditions. Besides that, mRNAs are immunogenic molecules, and activating immunity against mRNA may compromise the expression of target proteins and thereby thwart their therapeutic efficiency[34]. Considering sequence design is also essential to unleash the potential of mRNA. As a consequence, poor mRNA sequences can lead to unintended interactions leading to adverse events and possibly irAE[35], which may necessitate corrections to the therapeutic regimen. Finally, the overall success of the previous efforts to make mRNA deliverable is dependent on endosomal egress, as post-cellular uptake of mRNA molecules embeds a considerable amount within endosomes, rendering it non-functional[36]. To successfully implement mRNA therapies in clinical practice, these limitations need to be tackled.

The mRNA structure significantly influences translation efficiency and subsequent immune effects[37]. The inclusion of the 5′ capping sequence protects mRNA from exonuclease damage and promotes effective translation, whereas the poly(A) tail promotes ribosome recruitment and stabilizes mRNA[38]. Moreover, the secondary stem-loop or hairpin structures within coding sequences or unoptimized untranslated regions (UTRs) may interfere with the ribosome moving along the mRNA[38]. The design of 5′UTR should avoid the start codons, and incorporating the 3′ UTR of $\alpha$- and $\beta$-globin usually enhances mRNA stability and translation[38,39]. Still, mRNA's secondary structure is essential to confer protection from endo and exonuclease, which would prolong its half-life. Nevertheless, reports indicate that while high GC content can affect the secondary structure of mRNA, it also notably improves translation efficiency compared to the low GC counterparts[40]. Hence, achieving optimal antigen expression may involve algorithmic strategies to maximize the content of highly expressed GC nucleotides[41]. Moreover, when optimizing codons, it is important to select codons corresponding to abundant tRNA species to accelerate translation and avoid ribosomal pauses. Also, altering the structure and stability of mRNA can be achieved by substituting nucleosides or adding chemical groups, such as *N*1-methyl-pseudouridine or pseudouridine[42–44]. Further, nucleoside-modified mRNAs are less immunogenic, resulting in improved translation efficiency and protein synthesis.

Enhancing mRNA transfection efficiency by non-viral delivery tools mitigates the immunogenicity risks[28]. Several mRNA-based vaccine immunotherapies are undergoing clinical trials for cancer treatment due to their unique capability to trigger immune responses through translating cancer antigens and other immunostimulants[45]. One strategy is to use mRNA vaccines encoding TAAs, which induce reasonable immune responses and showed promise in preclinical studies and further progressed to clinical trials for oncology[46]. The development of mRNA vaccines encoding TAAs was planned to primarily express antigens in malignant cells[12,47]. Successful development of efficient TAA-encoded mRNA vaccines depends on antigen selection based on their expression levels and frequency, as well as tissue specificity in the target cancer[48].

In patients with advanced melanoma, the tetravalent BNT111 lipoplex encoding melanoma-associated antigens generated durable T-cell responses against particular TAAs (NY-ESO-1, MAGE-A3, tyrosinase, and TPTE)[49]. A further application of mRNA involves generating personalized vaccines targeting neoantigens determined from tumor exosome analyses[50,51]. Moreover, mRNA plays a role in cellular therapies, activating antigen-specific T-cells with patient-derived DCs or recognizing tumor antigens directly with T-cells encoded with chimeric antigen receptors[52].

#### 2.1.1. *Immunostimulant mRNA vaccines*

mRNA-encoding immunostimulants, typically cytokines or chemokines, are designated to mature and activate antigen-presenting cells, promote T-cell-mediated immunity, and counteract the immunosuppressive effects within TME[12]. Several mechanisms are involved in how cytokines contribute to cancer immunotherapy, including combating tumor growth and boosting antitumor immune responses. Cytokines such as interferon (IFN-$\alpha$) can directly exert anti-proliferative effects on tumor cells by activating JAK−STAT pathways and upregulating pro-apoptotic proteins like TRAIL[53]. Also, interleukins such as IL-2 and IL-12 are key players in modulating the TME by promoting Th1 differentiation and enhancing the cytotoxicity of immune cells against tumors[54]. Moreover, cytokines can mitigate immunosuppressive signals and angiogenic processes that promote tumor growth and metastasis within the TME. On the other hand, the release of chemokines into the TME can trigger the infiltration of tumor-destroying immune cells, including cytotoxic T lymphocytes[55]. Therefore, an effective antitumor immune response can be amplified by promoting specific chemokine expression that attracts immune cells to tumor sites. When chemokines are expressed in tumor cells, they become sensitive to chemotherapy, radiotherapy, and other immunotherapies, potentially enhancing their ability to fight tumors.

Immunostimulatory vaccines are preferably administered intratumorally[56] and intranodally[57,58] for improved accumulation, along with alternative routes such as intradermal and intravenous routes[56]. Clinical trials have shown great promise in combining mRNA vaccines, specifically those encoding immunostimulants and immune checkpoint inhibitors (ICIs), in treating melanoma[59]. Additionally, the introduction of mRNA-protamine complexes enhanced immunostimulatory properties and triggered consistent immune responses[60].

In 2018, Uchida and colleagues[61] proposed the introduction of safe and efficient dsRNA as an adjuvant to mRNA vaccine, leading to augmented synchronized antigen expression and immunostimulation within the same antigen-presenting cell. Uchida formulated mRNA hybrids with polyuridylic acid and full-length antisense RNA. The hybridized mRNA with poly A region successfully achieved efficient translation and robust immunostimulation. Notably, the immune response triggered by the polyuridylic mRNA hybrids was mediated through the Toll-like receptor (TLR3) and the retinoic acid-inducible gene. When TLR3 was activated, it recruited leukocytes into the TME and induced apoptosis by engaging natural killer (NK) cells and cytotoxic T-cells[62]. Compared to single-stranded mRNA, hybridized mRNA significantly improved specific cellular and humoral immune responses, as evident in DC activation.

A leading mRNA vaccine platform, RNActive®, developed by CureVac AG, is currently undergoing clinical trials specifically tailored for oncological applications[63]. RNActive® technology maintains unmodified nucleotides to ensure optimal immunogenicity with some enhancements[64]. These modifications include

highly translated untranslated regions, a poly-A tail, and algorithmic optimization of the open reading frame by including guanine and cytosine for superior protein expression[41]. Dual-component RNActive® vaccines are self-adjuvanted, containing both "naked" mRNA and mRNA-protamine complexes, thereby enhancing the immunostimulatory properties and triggering potent and consistent immune responses[60]. In the clinical context, CV9202, an RNActive®, demonstrated robust translation efficiency, resulting in elevated antigen expression and triggering immune responses against a spectrum of non-small cell lung cancer when combined with local radiotherapy. The combination aims to initiate a broader immune response cascade by spreading antigens, inducing immunogenic cell death, and amplifying the overall antitumor immune response[63].

### 2.1.2. *Personalized mRNA vaccines*

There have been inherent challenges associated with TAAs originating from fetal genes or tissue-specific proteins[65]. Restricted by thymic negative selection, T-cells generally fail to recognize TAAs due to their self-antigen status, inhibiting natural autoimmune responses and failing to initiate robust antitumor immunity[52]. Additionally, the induced immune response may also impact on-target noncancerous cells, as TAAs are not exclusive to tumor tissues. Moreover, the interpatient variability in TAAs expression, coupled with the tumor's ability to employ various escape mechanisms *via* downregulation of TAAs expression, further complicate the pursuit of an effective mRNA vaccine[52,66,67].

Alternatively, advanced sequencing tools have opened the doors to new eras of cancer immunotherapy with the identification of TSA and neoantigens. Neoantigens are the product of tumor-specific mutations recognized as foreign proteins, allowing them to circumvent the thymic negative feedback loop. For these reasons, neoantigens can elicit a more robust antitumor-immune response[68]. Neoantigens often emerge from mutations; therefore, new techniques that include leveraging personalized mutanome analysis and genomic comparison between healthy and cancerous tissues permit the recognition of neoantigens[51]. For instance, mRNA-4157 is an individualized neoantigen undergoing clinical evaluation for its efficacy in combination with Pembrolizumab in a phase 3 study (NCT05933577)[69].

Numerous challenges and technical hurdles need to be addressed to realize the potential of personalized cancer vaccines. The development process for personalized vaccines is particularly complicated, as seen in the identification and characterization of patient-specific neoantigens[70]. In this procedure, predicting immunogenic mutations through sequencing each patient's tumor genome is a prerequisite for formulating a tailor-made vaccine. Aside from that, another challenge lies in identifying reliable biomarkers to predict patient response. The search for specific biomarkers is hampered by the heterogeneous nature of the samples, limited sensitivity of the assays, and difficulty in validating the findings in a variety of patient populations[71]. Furthermore, regulatory and scalability hurdles impede the mass production of personalized mRNA cancer vaccines. The advancement of personalized vaccines from the bench to the clinical phase will require collaboration among researchers, clinicians, regulatory bodies, and industry stakeholders.

A primary tool for identifying neoantigens is Next-Generation Sequencing (NGS), which enables bioinformatics and MHC−peptide binding algorithms to predict neoantigen peptide (neopeptide) sequences. First, raw genomic data is acquired by whole exome sequencing (WES) of tumors and blood cells, which focuses on the exome, a protein-encoding gene region[72]. Identifying neopeptide typically involves data preprocessing and mapping the processed data to a reference genome to identify genetic changes. Afterward, mutant peptide sequences are determined and ranked according to their potential to bind with MHC molecules and interact with T-cell receptors. Prioritizing neoantigen candidates can be achieved by RNA sequencing, which mirrors peptide expression and abundance on the cell surface through RNA levels. These sequencing data are further processed by bioinformatic pipelines to identify peptide production and MHC molecule localization[73]. An example of such a pipeline is the Real-time Epitope Computation for Oncology (RECON) pipeline developed by Neon Therapeutics, which predicts therapeutically relevant neoantigen targets. This pipeline facilitated the generation of cancer vaccines targeting both unique neoantigens and those that are common among patients with similar cancer types[74].

Nevertheless, immunogenicity for the identified neopeptide-MHC complex is not guaranteed, and tools for recognizing the interactions with T-cell receptors are still in the early stages of development. Furthermore, reports from the Tumour Neoantigen Selection Alliance (TESLA) indicate that only a minority of predicted neoantigens are detectable by patients' T-cells, contrary to what most models predict[75]. Consequently, machine learning approaches have been employed to enhance prediction accuracy by analyzing and validating T-cell-stimulating neoantigens, as well as peptide features derived from immunopeptidomics. For instance, in 2021, Schmidt et al.[76] developed the Predictor of Immunogenic Epitopes (PRIME), integrating antigen presentation on HLA molecules and T-cell receptor recognition to rank neoepitopes by immunogenicity. Nevertheless, computational algorithms are less reliable in predicting MHC class II bindings[77]. Some algorithms enhance prediction accuracy by considering neopeptide foreignness and additional peptide features (such as cleavage and antigen transport). An example of the AI platform is EDGE, which analyzes tumor biopsy data to identify tumor-specific neoantigens and has been instrumental in developing personalized cancer vaccines like GRANITE-001[78,79]. Given the traditional prediction models may not consistently predict neoantigen presentation efficiency, even when supported by data showing evident RNA expression levels and translation efficiency, an alternative and direct approach to identifying neoantigens is through immunopeptidomics techniques relying on mass spectrometry. Immunopeptidomics rapidly identifies MHC class I associated neopeptides on the tumor's surfac [80].

### 2.1.3. *DC and T-cell-based vaccines*

There are two approaches to mRNA-based cellular immunotherapy: one involves pulsing DCs with mRNA derived from autologous tumors, and the other relies on generating T-cells with chimeric antigen receptors (CAR-T cells) that directly target tumor antigens. The high antigen presentation potential of DCs and their immune-modulation abilities make them the optimal tool for immunotherapeutic interventions. This technique involves the procedure of *ex vivo* loading mRNA encoding TAA into patient's DCs and re-infusing those cells to trigger antigen-specific T-cell responses (Fig. 3)[81].

Immunotherapy using CAR-T cells with binding domains that target cancer cells expressing TAA or TSA has emerged as a promising approach for managing tumors[82]. CAR-T-cell modification by mRNA and electroporation was proved to be safer and cheaper than viral vectors since this strategy grants temporary CAR expression without inducing genomic alterations[52,83].

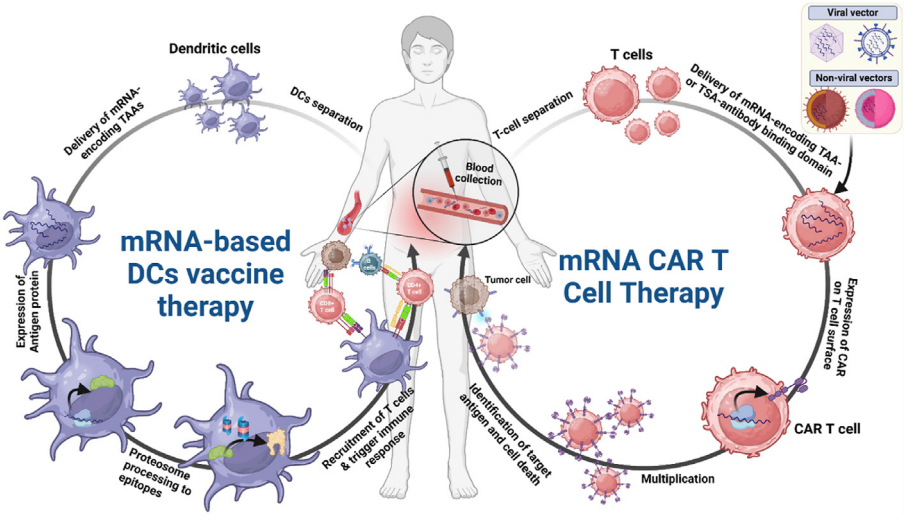


**Figure 3** The development of mRNA-based CAR-Ts and DCs immunotherapies. (Right) CAR-T cell therapy has evolved by delivering mRNA-encoding antibody binding domain (CAR) to patient-isolated T-cells, which are then expressed, multiplied, and transferred back to the patient to recognize and destroy the malignant cells. (Left) DC vaccines have been developed by introducing mRNA-encoding TAAs into patient-isolated DCs, which are transferred to the patient after expression and epitope presentation for tumor recognition and triggering cytotoxic immune responses. Figure was made by Biorender.com.

Moreover, recently, in the BNT211-01 trial (NCT04503278), CAR-T cells expressing CLDN6 binding domains were investigated for tolerability and potential efficacy in solid tumors[84]. With a 67% disease control rate, this therapy has shown promising efficacy in treating relapsed or refractory CLDN6-positive solid tumors. Despite its manageable toxicity, the vaccine caused the cytokine release syndrome in 46% of patients. However, an earlier phase 1 study of six patients with refractory metastatic pancreatic cancer treated with recurring infusion of electroporated mRNA CAR-T cells targeting mesothelin showed that the treatment was effective with no off-tumor side effects or cytokine syndrome (NCT01897415)[85]. According to the trial, disease stabilization was observed in two patients, with 3.8 and 5.4-month progression-free survival. Also, another patient was reported to have his metabolic active volume declined by 69%, evidenced by the completely reduced uptake of fluorodeoxyglucose in all liver lesions following one month of treatment, even though primary pancreatic lesions were not affected.

Cancer therapy has advanced with the advent of CAR-T, but a few obstacles remain to optimize the treatment's efficiency. Post-CAR-T therapy, tumor cells may enter a state of antigen escape, where they lose the ability to express the target antigen[86]. Thus, the proposal of the multi-antigen targeting approach could potentially resolve antigen escape; for instance, CD19 and CD22 were targeted in NCT03287804. However, the success of this approach is not guaranteed, since in this phase 1 trial, the proliferating ligand (APRIL) CAR showed only modest clinical responses despite manageable toxicity in people with refractory multiple myeloma[87]. Nonetheless, cytokine release syndrome in five patients was grade 1 and there was no neurotoxicity reported. On the other hand, among eleven patients, only five showed clinical responses, including one who showed a very good partial response. There was observed mechanistic inadequacy of the APRIL CAR, including reduced IL-2 secretion, impaired IFN signaling, and lack of sustained tumor control. This suggests that T-cell-expressing APRIL had poor binding affinity for soluble B-cell maturation antigen, compromising the function of CAR.

*In situ* vaccination presents an opportunity to overcome the drawbacks of generating *ex vivo* CAR-T or DC vaccines using mRNA. This approach bypasses lengthy and labor-intensive *ex vivo* procedures by direct delivery of mRNA-encoding tumor antigens or CAR constructs into the patient. Rather, these constructs can be expressed *in vivo*, allowing them to be successfully targeted at DCs for antigen presentation or to be directly modified within T-cells[88]. This method ensures sustained antigen expression, addressing the shortcomings such as limited T-cell response and resistance experienced with *ex vivo* treated DCs, as well as eliminating the necessity of lymphodepletion[89]. Moreover, *in situ* development avoids the adverse events associated with *ex vivo* redirected CAR-T therapies, including cytokine storm and immune effector cell-associated neurotoxicity syndrome[89]. However, safety concerns remain around mRNA expression within off-target cells[90]. Another advantage of *in situ* vaccine development is the simplified treatment regimens and standardizable manufacturing at reduced production costs[89]. Nevertheless, challenges remain in optimizing delivery systems to guarantee targeted and efficient delivery of mRNA while enhancing immunogenicity to improve antitumor responses.

### 2.2. *Synthetic therapeutic oligonucleotides (STOs)*

RNAi has proven a highly effective approach in cancer therapy with the advantages of precise and targeted gene silencing, cost-effectiveness, and diverse treatment options. Unlike traditional therapies such as chemotherapy, RNAi can concurrently inhibit multiple cellular pathways involved in tumor progression. Ongoing clinical research into RNAi-based cancer therapies has revealed their potential to revolutionize cancer treatment by targeting druggable and undruggable therapeutic targets, as presented in Fig. 2. The evolution of STOs, including Antisense oligonucleotides (ASOs), aptamers, and siRNAs, provides an avenue for RNA degradation and splicing modulation mechanisms (Fig. 2). Preclinical development of several STOs is underway, and more than a hundred STOs are currently experiencing the

clinical phase of development. Despite the promise of STOs, achieving optimal STO hinges on conquering a set of extracellular and intracellular constraints, including cellular uptake and escape from endolysosomal compartments, which call for the development of an effective platform for delivery[91].

#### 2.2.1. *Small and short hairpin interfering RNAs (siRNAs & shRNA)*

The concept of RNAi was first established by Fire and Melo in 1998 to degrade the target mRNA using dsRNA, which led to blocking gene expression unexpectedly[92], which credited them with the Noble Prize in Physiology or Medicine in 2006. These dsRNAs are short noncoding RNA duplexes of 30 to more than 100 base pairs. As part of the RNAi process, Dicer enzyme cleaves dsRNA into ~ 20 nucleotides siRNAs. Once internalized into a cell, siRNAs can directly embed themselves in the RNA-induced silencing complex (RISC) without being subjected to any subsequent processing. Meanwhile, shRNAs exhibit distinct structural configurations and form stem-loop structures. Therefore, Dicer activation for shRNAs is necessary before participation in RNA interference. Next, shRNAs bind specifically to endonuclease argonaute 2 (Ago2) in the RISC assembly, converting it into a single antisense strand. shRNAs and siRNAs serve as guides to the target mRNA, leading to effective gene knockdown. This silencing strategy can suppress desired genes in a highly efficient and precise manner, as the antisense strand often fully complements the coding region of the target mRNA.

siRNAs have undergone several modifications to enhance their serum stability and deliverability. This includes tuning the sugar moiety, phosphate structure, RNA bases, and 3′ overhangs. To enhance their stability in serum, modifications at the 2′-O position of the ribose are typically applied, such as adding methyl, methoxyethyl, and fluoride[93,94]. A further modification to the 2′ position involves introducing azido-modified cytidine or guanosine or introducing gemcitabine into siRNAs, which were reported to boost nuclease resistance and knockdown efficacy[95,96]. It has also been reported that masking 2′-OH with acyloxymethyl facilitated cellular uptake and enhanced gene suppression[97]. Moreover, modifying the 3′ overhangs with 1-deoxy-D-ribofuranose increases nuclease resistance and knockdown efficiency. It was also reported that gene silencing activity could be augmented by replacing the traditional phosphate in the backbone structure with phosphorodithioate, boranophosphate[98], or triazole backbone linkages[99] has also been shown to augment siRNA gene silencing activity. According to Huang et al.[100], further modifications to L- and D-isonucleosides may affect thermal stability differently, with D-isonucleotides having a less pronounced effect than L-isonucleotides while increasing the serum stability of siRNA. Further, the presence of D- or L-isoNA at the 3'-terminus of the sense strand notably promotes gene knockdown.

Recently, modifying siRNAs with GalNAc presents a highly efficient delivery strategy to hepatocytes, exploiting the presence of highly expressed Asialoglycoprotein receptors to facilitate the uptake[101]. Following this breakthrough, multiple Phase III clinical trials have resulted in FDA approval for the first GalNAc−siRNA conjugate (Givosiran). Efforts to improve the stability of siRNA molecules include introducing phosphorothioates at the termini of siRNA molecules[102,102], forming small and readily synthesizable conjugates of siRNA and GalNAc that target asialoglycoprotein receptors in hepatocytes to reduce dosage and off-target effects[103]. GalNac linker arms can be naturally degraded by endosomal glycosidases, releasing siRNA into the cytoplasm. These approaches are under clinical investigation and are also being developed by leading USA Biotech companies[104].

The clinical relevance of siRNAs gained momentum after the FDA approved Patisiran in 2018 when siRNA was accepted for treating hereditary polyneuropathy. A growing number of siRNA drugs have been approved over the past five years, showing their therapeutic potential[105]. Even though there is growing evidence that sequence-specific antitumor siRNA could be designed for cancer treatment, several challenges remain, including concerns about their cost-effectiveness, deliverability, stability, off-target effects, poor biodistribution, and pharmacokinetics[106]. siRNAs can be delivered into cells in several ways, whether naked, conjugated molecules or complexed with a vector.

In the quest to induce therapeutic antitumor immune responses, researchers strategically target specific molecular pathways, including anti-inflammatory cytokines, immune checkpoint proteins, and pivotal immune signaling molecules. Several studies show great promise for potential applications in TGF-$\beta$-targeting siRNA in both melanoma and glioblastoma[107,108]. The delivery of siRNA in combination with the Trp 2 peptide and cytosine-phosphorous-guanine oligodeoxynucleotides (CPG ODN) has significantly enhanced vaccination efficacy in melanoma through reducing regulatory T-cell levels, increasing $CD8^+$ T-cells infiltration[107]. One reason to include CPG ODN as an adjuvant is to leverage bacterial DNA sequence patterns for TLR9 activation cytokine production, thereby initiating a sustained immune response[109,110]. Additionally, modifying siRNAs with CpG ODNs was reported to allow them to be delivered to specific cells independently of a delivery agent[111]. In glioblastoma, coadministration of temozolomide and TGF-$\beta$ siRNA has achieved an effective antitumor immune response[108]. Indirect methods have been used to suppress TGF by silencing $\beta$-catenin[112,112], or by knockdown of $\beta$-catenin to enhance $CD8^+$ T-cell infiltration in hepatocellular carcinoma when used with anti-PD-1 treatment[113].

Immune checkpoints such as PD-L1 (programmed death ligand-1) or NOTCH in DC and CTLA-4 in T-cells can also be targeted by siRNA. Silencing these checkpoints has increased infiltration of tumor-killing $CD4^+$ and $CD8^+$ T-cells and reduced levels of immunosuppressive cells[114−116]. CD47 is another potential checkpoint protein target for siRNA and plays a vital role in regulating phagocytosis[117]. In a recent communication by Yu group, the antitumor cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors were shown to be compromised by PDL-1 overexpression. The researchers have demonstrated that siRNA-mediated PD-L1 knockdown in tumor cells has enhanced the effect of CDK4/6 inhibition and facilitated the infiltration of cytotoxic T lymphocytes, thus suppressing colorectal cancer growth efficiently *in vitro* and *in vivo*[118].

There is an increasing interest in manipulating gene expression in DCs *via* RNAi approaches and using siRNA to minimize the immunosuppressive effects of TME in cancer therapy. This includes the release of inhibitory enzymes, interleukins, and other essential proteins and receptors[119]. A crucial immunosuppressive enzyme is Indoleamine 2,3-dioxygenase, which contributes to multiple negative immune responses, primarily suppressing T-cells and NK cells or activating Tregs [120,121]. Therefore, NK cell infiltration was noted at the tumor site following the administration of shRNA to silence this dioxygenase in mice grafted with ovarian cancer[122]. Similarly, dioxygenase expression in splenocytes and lymphocytes was effectively blocked by siRNA, contributing to reduced apoptosis of $CD8^+$ and $CD4^+$ T-cells, along with enhanced response to melanoma antigen[123].

Additionally, siRNA targeting ubiquitin editing enzyme A20 or negative regulatory receptors like DIgR2 in DCs enhances antigen-specific T-cell reactions and suppresses regulatory T-cells. Similarly, dioxygenase expression reduced the apoptosis of $CD8^+$ and $CD4^+$ T-cells with improved response to melanoma antigen[123]. Additionally, siRNA targeting ubiquitin s-has been applied to DCs triggers ubiquitin-editing enzyme (A20) or negative regulatory receptors like DIgR2 (dendritic cell-derived immunoglobulin receptor 2) in DCs enhances antigen-specific T-cell reactions and suppress regulatory T-cells[124,125]. Bcl-2 siRNA has been applied to DCs triggers Rig-I (retinoic acid-induced protein I), ultimately resulting in the expression of IFN and tumor apoptosis in melanoma[126].

It is becoming evident that RNA can target negative regulators engaged with tumor progression. For example, selective shRNA-mediated knockdown of the negative regulator suppressor of cytokine signaling 1 (SOCS1) has improved antigen-specific antitumor responses[127]. siRNA has the potential to eliminate the effects of TAMs on tumor progression and metastasis by targeting the M2-like receptor of colony-stimulating factor-1 and chemokine receptor-2 on monocytes[128,129]. This further expands RNAi in cancer research.

#### 2.2.2. *MicroRNA*

MiRNAs are non-coding RNAs of around 18−22 nucleotides that control mRNA stability and degradation through the RNAi machinery. Primary miRNAs are the initial product transcribed from genomic DNA, but they undergo cleavage by the Drosha subunit complex to form precursor miRNAs (pre-miRNAs). These pre-miRNAs are then transported to the cytoplasm, where DICER processes it to miRNA duplexes. Upon interaction of miRNAs with RISC, the duplexes are unraveled, forming miRISC that will then interfere with mRNAs, typically binding to the 3′ untranslated region. Unlike siRNA, miRNAs may induce gene silencing through slicer-independent pathways.

MiRNAs notably foster the crosstalk between tumor and immune cells within TME, influencing immune cell functions and playing a demonstrated role in evading immune reactions. Additionally, these miRNAs can indirectly influence macrophage polarization by modulating signaling pathways like PI3K/AKT and PTEN/PI3K$\gamma$. For instance, miR-301a and miR-103a secreted in tumor exosomes were involved in regulating TAM polarization to M2, contributing to tumor progression and immunosuppression[130,131]. This process creates a feedback loop where M2-polarized TAMs further enhance cancer progression and metastasis. In addition, miR-29a-3p, miR-21-5p, miR-29a, miR-21, miR-155-5p, miR-375, and miR-321a produced by M2 cells were implicated in modulating NF-$\kappa$B pathway and accordingly their cytokine production, and cancer cell invasion. Tumor-suppressing miRNAs, like miR-BART cluster, can directly regulate antitumor immune responses by simultaneously blocking key checkpoints like PD-1 and PD-L1 in the TME[132]. Also, several miRNAs, including miR-301a, miR-22, and miR-221, could affect cytokines produced from DCs. Moreover, MDSC was found to proliferate in response to STAT3 pathways activation by miR-155 and miR-21, thereby interfering with T-cell responses.

Emerging miRNA treatments, including INT-1B3 (miR-193a-3p), miR-10b, and MesomiR-1, show promise in treating advanced and metastatic tumors[133−135], as well as mesothelioma[136]. Cobomarsen is another promising miRNA for treating cutaneous T-cell lymphoma by inhibiting miR-155, showing promising efficacy and safety in the phase 1 trial (NCT02580552)[137]. In this trial, those patients who received Cobomarsen intratumorally demonstrated a reduction in lesion index scores by more than 50%, which was maintained during the study, along with evidence from histological and gene expression analyses. The Cobomarsen injection was well tolerated and had no clinically significant side effects, except for one patient who experienced grade 3 pruritus.

#### 2.2.3. *Antisense oligonucleotides*

Antisense oligonucleotides (ASOs) are short single-stranded synthetic oligonucleotides, highly modified to selectively bind *via* complementary base-pairing to RNA for treating specific disorders[138]. Usually, ASOs are formed of either modified DNA or RNA bases or both. The development of ASOs took a significant step forward when Fritz Eckstein introduced phosphorothioate modifications in 1966[139]. The procedure used sulfur to substitute one oxygen in the phosphate group, enhancing the hydrophobicity of ASO and increasing the resistance to phosphodiesterases. The modifications also enhanced the affinity for albumin binding and thereby improved stability and pharmacokinetics[140].

The selectivity of the ASO to the opposite strand permits its interference with mRNA processing[141]. When the ASO binds to complementary bases in pre-mRNA, it prevents splicing activities, hampers the attachment to ribosomal units, and blocks the protein translation because triggering RNase-H leads to mRNA cleavage[142]. Ultimately, this cleavage degrades the targeted RNA, resulting in an effective gene knockdown. Pre-mRNAs and long noncoding RNA are the targets of RNase H1-dependent ASOs, which are inaccessible to siRNA. However, there are also RNase H-independent ASOs, commonly known as steric blocking ASOs, which directly obstruct or enhance translation or splicing by their steric blocking ability[143].

These ASO-based therapeutic tools are highly promising for treating various genetic disorders and cancers by targeting specific mRNA molecules and modulating their expression. Mipomersen, Inotersen, and Volanesorsen are three FDA-approved ASO gapmers that are made up of a central DNA segment flanked by modified RNA segments that act selectively when coupled to RNA sequences of the target[144]. In 2016, intrathecal injection of Nunsinersen was the first 2′-methoxyethyl RNA-based ASO to gain FDA approval for spinal muscular atrophy[145]. As yet, no approval has been granted for ASO for cancer treatment, though three ASOs containing DNA bases have been assigned orphan drug status, including oblimersen, Cobomarsen, and PNT2258[142].

ASOs targeting specific vital cellular processes such as JAK2/STAT3 and PI3K/AKT axes and other potential immunosuppressive components might show promising effects in cancer treatment[146]. Upregulation of Bcl-2 and Akt was correlated with human chronic lymphocytic leukemia[147]. Additionally, targeting Bcl-2 with ASOs has demonstrated antitumor efficacy and longer survival time in lung cancer xenograft models[148]. Also, targeting immune-inhibitory surface ectoenzyme, such as CD39, by ASOs reduced the production of immunosuppressive adenosine and ultimately decreased tumor growth[149]. Moreover, the suppression of metadherin expression with locked nucleic acid-modified ASO is linked to the suppression of cytotoxic T-cell depletion and suppression of cancer growth[150].

### 2.3. *Aptamers*

Aptamers are opening up new possibilities for cancer therapy due to their unique intrinsic properties, such as small size and

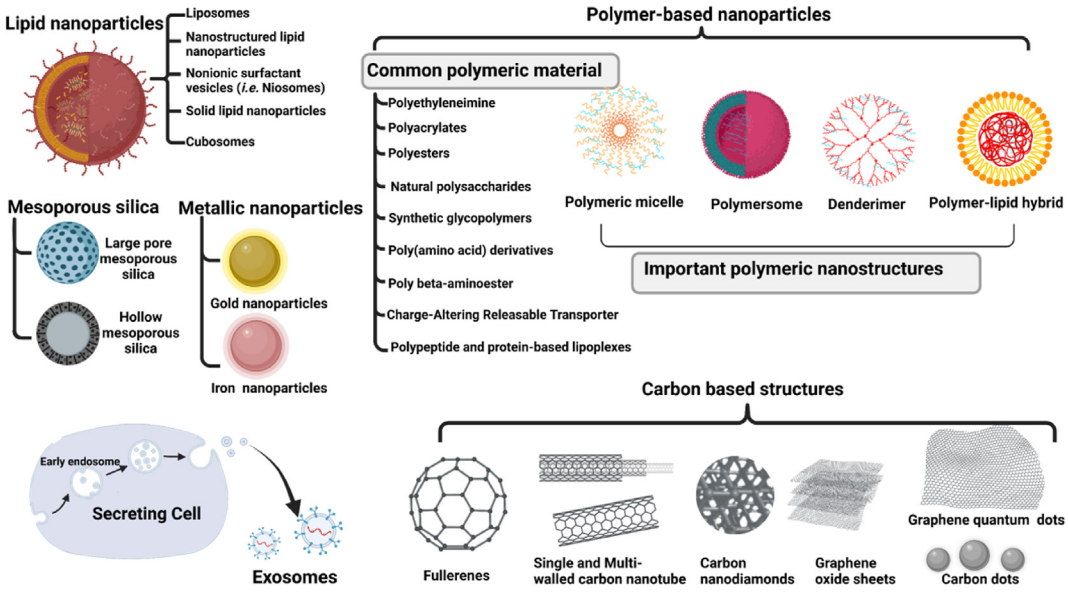


**Figure 4** Nanoplatforms for RNA delivery. Figure was made by Biorender.com.

chemically adaptable nature, for better stability and specificity for one or more molecules[151,152]. An aptamer is formed of a single strand of nucleic acid, either RNA or DNA, which folds into a 3D structure, being able to recognize and bind to a target molecule[152]. In addition, aptamers could be tailored to serve as dual-specific or multi-specific agents, capable of targeting tumor cells and enhancing antitumor immune responses[153].

There may be instances where standard immunotherapies cannot effectively fight resistant tumors. Aptamers have demonstrated remarkable potential to boost the immunotherapeutic response in various malignancies by modulating immune responses within TME[154]. Aptamers may also target biomarkers associated with drug resistance, potentially exposing cancer cells to therapeutic intervention. They can also be used as delivery agents for cytotoxic drugs, bypassing multidrug resistance mechanisms, including P-glycoprotein transporters, and delivering drugs directly into cancer cells[155,156]. Future perspectives include developing personalized aptamers that target specific molecular pathways with unique sequences specifically designed for individual patients, aiming to maximize therapeutic response and minimize adverse effects[157].

## 3. Nanoscale platforms for RNA delivery

Recent advances in nucleic acids and macromolecules-based therapies have demanded the development of diverse strategies to address delivery-related challenges. Still, designing effective delivery tools for cancer tissues entails overcoming robust biological barriers created under the effects of TME and loss due to interaction with serum proteins or extracellular enzymes[158]. Cellular entry of these molecules is also prevented by electrostatic repulsion with the cancer cell membrane, and the escape from the formed endosomes is a major impediment to release at their target[159]. Since the advent of mRNA lipoplexes, recent and future studies are pursuing promising non-viral components of the delivery systems. The quest for an optimal vector in RNA delivery has led to the exploration of various nanomaterials, such as lipid-based, polymeric, and inorganic excipients. Therefore, in this section, we highlight the significance of polymers-, lipids-, exosomes-, metal, and silica-based delivery systems (Fig. 4). Each delivery system possesses its benefits and drawbacks, as outlined in Table 2[160-196].

### 3.1. *Lipid-based systems*

#### 3.1.1. *Liposomes and liposome-like nanoparticles*

Lipid-based nanovesicles (liposomes or LPS) are commonly explored for RNA delivery and are formed by encapsulating hydrophilic or lipophilic molecules within the lipid bilayer structure or entrapped in aqueous space. The lipoplex encapsulating materials involve cationic, ionizable, or zwitterionic lipids, as well as phospholipids and helper lipids, which are incorporated into the formulation to promote cellular uptake, membrane destabilization and, therefore, enhance nucleic acid delivery (Table 3)[162-199]. More recently, other lipid nanoparticles (LNPs), which can enclose molecules in the non-aqueous interior of liposome-like particles (LLPs) or micellar structures, have been found promising as a safe and reliable means of delivering RNA tools. Fig. 5 presents the differences between LPS and other lipid-based delivery systems.

Cationic lipids, like DOTAP, play a crucial role by forming electrostatic bonds with negatively charged RNAs, promoting binding to cell membranes and facilitating endosomal escape for cargo release into the cytosol. RNA payloads that are highly encapsulated in these lipoplexes are protected against breakdown by circulatory RNases. In clinical trials, cationic DOTAP-based LNPs are being investigated for their efficacy in delivering mRNA-DCs vaccine for recurrent glioblastoma cases[197].

Furthermore, targeting RNA lipoplexes to solid tumors relies on the enhanced permeability and retention (EPR) effect, which is not universal across all solid tumor types[211]. The short circulation half-life of lipoplexes is attributed to reticuloendothelial absorption, coupled with increased immunogenicity that results from phagocytosis. Moreover, cationic lipids may nonspecifically bind to other targets, causing off-target effects contributing to systemic toxicity. Therefore, balancing the positive charge for effective encapsulation with the neutral or nearly neutral surface charge under physiological conditions is critical to achieving circulatory

**Table 2** The advantages and drawbacks of RNA delivery systems.

| Delivery system | Advantage | Drawback | Ref. |
|---|---|---|---|
| Lipid-based systems | 1. Functionality of certain lipids surfaces<br>2. PEGylated lipid nanoparticles are non-immunogenic and exhibit longer circulation time<br>3. Synergistic co-delivery of genes and pharmaceuticals<br>4. Protection against RNA degradation<br>5. Easily produced by microfluidics and can be scaled up<br>6. Protein corona could enable the targeting of specific organs | 1. Cytotoxicity of cationic lipids<br>2. Limited transfection efficiency<br>3. Neutral lipid nanoparticles have low RNA loading capacity and limited stability<br>4. Serum proteins might impair targeted RNA delivery<br>5. Production costs and complexities<br>6. Inherent low biostability and short circulation time | 160−167 |
| Polymer-based systems | 1. Low immunogenicity<br>2. Protection against RNA degradation<br>3. Size, shape, and surface are modifiable for drug or gene delivery<br>4. Improve intracellular uptake by interaction with the cellular membrane | 1. Non-biodegradability of some polymers<br>2. Cytotoxicity and hemolytic activity of cationic polymers, especially PEI<br>3. Low loading capacity<br>4. Slow or incomplete cargo release | 168−173 |
| Exosomes | 1. Biocompatibility and non-immunogenicity<br>2. Targeted delivery<br>3. Ability to cross biological barriers, such as the blood−brain barrier<br>4. Exosomes could camouflage the phagocytosis process | 1. Lack of FDA approval<br>2. Heterogeneity in size and cargo content<br>3. Variability in encapsulation efficiency<br>4. Limited yield<br>5. Scalability and cost are challenging.<br>6. Storage instability | 174−179 |
| Organic-inorganic mesoporous silica nanohybrids | 1. High surface area and pore volume, allowing for high loading capacity<br>2. Versatility in surface decoration<br>3. Able to co-load different active agents with targeting agents<br>4. Synergistic co-delivery of drugs and genes<br>5. Protection of loaded RNA from degradation<br>6. DMSONs show better biocompatibility and biodegradability | 1. Traditional types are not suitable for RNA delivery<br>2. Low biocompatibility and potential toxicity<br>3. Complex and time-consuming preparation process.<br>4. Upon long-term storage, nanoparticles tend to aggregate<br>5. Clearance by the immune system | 180−184 |
| Carbon-based nanostructures | 1. High surface-to-volume ratio<br>2. CNTs have a high aspect ratio<br>3. Tunability for enhancing solubility or targeted gene delivery<br>4. Protect genetic material and improve transfection efficiency<br>5. Biocompatibility<br>6. Temperature-responsive delivery<br>7. Magnetoporation can potentially enhance CNT delivery | 1. Not biodegradable and toxicity concerns<br>2. Poor water dispersibility<br>3. Impaired pharmacokinetic properties<br>4. Instability of specific structures, such as quantum dots | 185−191 |
| Metallic nanoparticles | 1. Biocompatibility<br>2. Easiness of surface decoration for targeted delivery<br>3. Inherent targeting properties of magnetic nanoparticles<br>4. Enhanced transfection could be obtained by magnetofection<br>5. Photoacoustic imaging and photothermal therapeutic properties of gold nanoparticles | 1. Not biodegradable<br>2. Nanoparticle aggregation concerns upon long-term storage<br>3. Increased cost and production hurdles<br>4. Safety concerns with repeated administration | 192−196 |

stability, which can be addressed by incorporating helper lipids into formulations[166]. Fortunately, advances in smart ionizable lipids possessing weakly basic moieties may provide a potential solution to these challenges.

Ionizable lipids are pH-responsive and enhance transfection efficiency and target endosomes. They can protonate and deprotonate in response to pH changes. Under physiological pH, they retain a negative to neutral charge, thereby reducing the incidence of cytotoxic effects on blood cells and improving the system's biocompatibility. As soon as these lipids enter the endosomes, protonation occurs under acidic circumstances, creating a positive charge that facilitates their endosomal escape.

Moreover, ionizable lipids offer a set of remarkable features for RNA delivery. The lipid 1,2-dilinoleyloxy-*N*,*N*-dimethyl-3-aminopropane (DLin-DMA) with its linoleic acid chains increases gene knockdown efficiency[212], as the less saturated fatty acid exhibits superior efficiency in intracellular nucleic acid delivery. Also, the gene-silencing efficiency is linked to the p$K_a$ of ionizable lipids; hence, it is essential to maintain a p$K_a$ range of 6.2 to 6.5[213,214]. The ionizable lipid DLin-MC3-DMA has been successful in liver-targeted gene silencing[214]. It is a major constituent of the FDA-approved formulation of Onpattro®[215,216].

Other types of lipids have been developed, such as zwitterionic and biodegradable lipids, aiming to boost potency,

**Table 3** Roles of different lipid types in lipid-based nanoformulations.

| LNPs component | Role | Ref. |
|---|---|---|
| Cationic lipids (*e.g.*, DOTMA, DOTAP, DODAC) | 1. Encourage electrostatic interactions with negative phosphates in nucleic acid backbones, yielding higher encapsulation efficiency.<br>2. Cationic lipids are directly involved in interactions with cell membranes, facilitating endosomal uptake.<br>3. Interact with the anionic lipids in endosomal membranes to facilitate nucleic acid release to the cytosol. | 162,165 |
| Ionizable cationic lipids (*e.g.*, DODAP, DLin-MC3-DMA and DLin-KC2-DMA) | 1. Their neutral charges at physiological pH reduce toxicity and immune responses.<br>2. Enhance the endosomal escape by responsive protonation under acidic environments.<br>3. Grant longer circulation times compared to cationic lipids.<br>4. Their apparent p$K_a$ value is critical to attain efficient transfection of RNA molecules | 160,161 |
| PEG-lipids (*e.g.*, PEG-c-DMG, PEG-DSPE) | 1. PEG positively affects LNP particle size and polydispersity, eliminating LNP aggregation.<br>2. Contribute to enhanced particle stability during preparation and storage.<br>3. Promote RNA encapsulation and transfection efficiency.<br>4. PEG could camouflage the immune responses, prolong circulation half-life, and promote *in vivo* distribution of the loaded RNA. | 162−164 |
| Helper lipids<br>Cholesterol | 1. Forms liquid-ordered phase in LNP membranes, leading to improved membrane integrity.<br>2. Contributes to LNP structural stability and prevents cargo leakage from the core by enhancing membrane rigidity. | 162,165 |
| Helper lipids<br>Phospholipids (*e.g.*, DSPC, DOPE) | This lipid type helps improve encapsulation efficiency, stability, membrane fusion, and intracellular RNA delivery. For example:<br>1. DSPC stabilizes and improves the intracellular delivery of LNPs.<br>2. DOPE promotes hexagonal phase formation, facilitating transfection efficiency and enhancing expression of the delivered mRNA | 162,198,199 |

biodegradability, and safety. Zwitterionic lipids with pH-sensitive properties have imparted potent protein expression *in vivo* as a consequence of bypassing endosomes. On the other hand, biodegradable lipids, such as SM-102, emerge as more effectively cleared with less toxicity alternatives, whereas 244cis offered several advantages for Cre recombinase enhanced potency, safety, and repeated administration, enabling substantial target protein expression in the lungs[217].

The lipid-like material (C12-200)-based lipidoid has significantly reduced the siRNA doses for liver multiple gene silencing by 100-fold in non-human primates[218]. Among these lipidoids, cKK-12 demonstrated high potency in Factor VII knockdown by siRNA superior to DLin-MC3-DMA nanoparticles[219]. Some lipids play a role in anti-cancer immunotherapy that goes beyond serving as carriers, having a synergistic therapeutic effect. Lipidoids, with cyclic amino head, triggered the STING (Stimulator of Interferon Genes) pathway that upregulates several immunostimulatory factors like IRF7, CXCL10, and IFN-$\beta$, necessary to potentiate T-cell reactions[220].

To improve biodistribution patterns, LNPs can be surface decorated by apolipoprotein E (ApoE) that mimics physiological lipoproteins in their targeting strategy to hepatic tissue, which act naturally based on affinity to LDL (low-density lipoprotein) receptors on hepatocytes[221]. ApoE improved cellular uptake[219], and enhanced the escape of siRNA confined within endosomal membranes, steering the delivery to the liver[221]. It was therefore noted that the formation of a protein corona affects LNP recognition and immune cell interactions, as well as their delivery and biological fate[222,223]. This has inspired Dilliard et al.[224] to formulate targeted LNPs that are precise in delivering mRNA to target organs in mice by designing lipid structures with the selective organ targeting (SORT) molecule. SORT-based LNPs displayed unique and specific organ-targeting outcomes due to their tailored alkyl tail and headgroup characteristics and their interactions with plasma proteins. For example, they showed vitronectin as a key protein interacted favorably with 18:1 DOTAP as a SORT molecule and steered mRNA delivery from hepatic tissues to the lungs.

Lipopolyplexes exert different structural assemblies from the conventional LNPs to address challenges posed by lipoplexes. A redox-responsive lipopolyplex has been developed, using dendrimer (G0-C14), cysteine-based poly(disulfide amide), PEG-lipids (DSPE-PEG 2000 and DMPE-PEG 2000), to deliver p53 mRNA to treat liver and lung cancer[225]. The PEG lipids have relative stability with sustained degradation, providing longer circulating time and improved pharmacokinetic properties[226]. Meanwhile, DMPE-PEG is rapidly de-PEGylated, which makes it more prone to be absorbed by cells.

Despite the previously outlined benefits, lipoplexes pose various challenges, including complex synthesis, potential toxicity, and their large vesicular size, which interferes with diffusion and biodistribution to target cancer tissue. The quest to provide controlled particle size with a high polydispersity index and homogeneity opened an opportunity for applying microfluidic mixing devices. Microfluidic fabrication of LNPs enables an exceptionally high to full siRNA encapsulation, making the technique a potential technology for the biopharmaceutical industry[167].

### 3.1.2. *Solid lipid nanoparticles and nanostructured lipid carriers*

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) differ primarily in their interior lipidic core composition and loading capacity. In fact, SLN has limited capacity due to the presence of a solid crystalline matrix that limits the loading of molecules to the core structure. Meanwhile, the NLC, which is created by combining liquid and solid lipids, produces irregularities during its formation, which renders it easier for pharmaceuticals to be incorporated into the matrix[207,227].

Advances with DDAB-based cationic SLNs carrying siRNAs targeting EphA2 receptor tyrosine kinase (siEphA2) have yielded

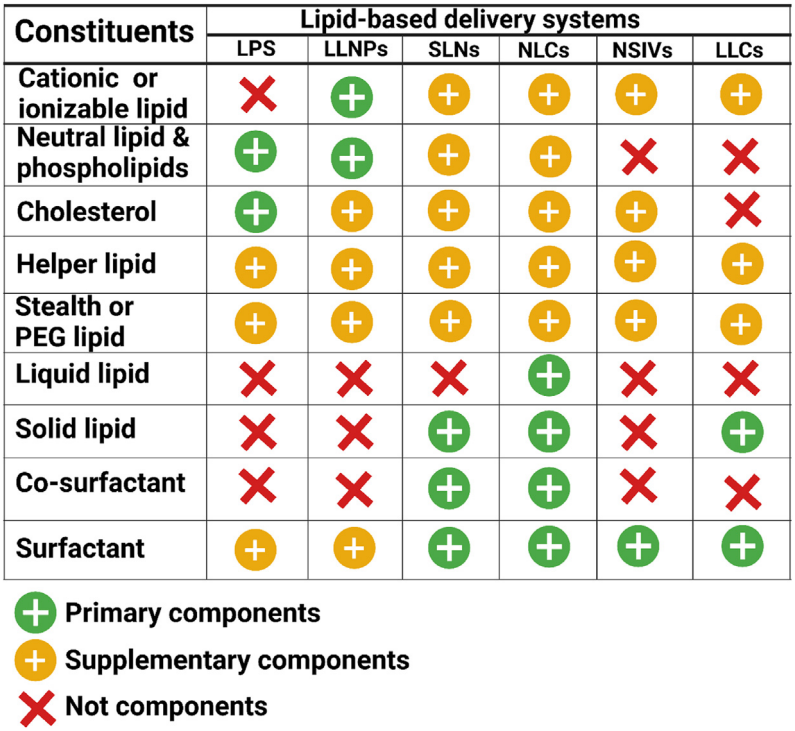


**Figure 5** The constituents of different types of lipid-based delivery systems. Constituents were selected and sorted according to the literature[148,162,166,198,200–210]. Figure was made by Biorender.com.

positive results against prostate cancer[228]. As well, cationic NLCs have been tested for miRNA delivery to treat cancer. Piao et al.[208], for example, revealed that the delivery of miR-107 by cationic NLCs significantly inhibited the growth, migration, and invasion of head and neck squamous cell carcinoma. Nevertheless, the toxicity of cationic NLCs and SLNs limits their potential. Alternatively, neutral NLCs provide a safer alternative that is less toxic, although their encapsulation efficiency can be compromised.

### *3.1.3. Nonionic surfactant vesicles*

Nonionic surfactant vesicles (NISVs) are categorized into cationic niosomes and SPANosomes that have shown potential for RNA delivery[202,203]. The formation of nioplexes by complexing nucleic acids with cationic niosomes is crucial for delivering nucleic acids[229]. Span 20 and cholesterol have proven effective for creating NSIVs, suggesting a future direction for mRNA vaccine formulation in cancer immunotherapy. Nioplexes have a protective shield, ensuring that the RNA remains stable and intact while transported to the target site[230]. The condensation of RNA on the surface of cationic niosomes is a critical aspect of the nioplexes formulation proces[231], enhancing their loading capacity and genetic material delivery to target cells.

A further advantage of nioplexes is controlled release, optimizing transfection efficiency and nucleic acid performance[204]. Moreover, nioplexes' compositions are versatile. This nanocarrier can be tailored to meet specific delivery requirements, including their entrapment efficiency, size, surface charge, and release properties[232,233]. The development of advanced machine learning techniques has greatly aided the optimization process of niosomal drug delivery systems, reducing both laboratory time and cost[234].

A novel synthesized spermine-based cationic lipid was incorporated to form cationic niosomes for the delivery of green fluorescent protein-encoding pDNA. Notably, these nioplexes demonstrated high transfection efficiency, serum stability, and safety *in vitro*, along with physical stability for at least 1 month at 4 °C[231]. However, in a comparative study, the nioplex-based delivery system showed the lowest uptake by cells compared with lipoplexes and polyplexes[203].

Microfluidic mixing was employed in 2017 to formulate NSIVs composed of Tween 85 and cationic charge from DDA, showcasing the ability of NISVs to transport and transfect GFP siRNA into cells. In 2012, Zhou and colleagues developed SPANosomes, constituted mainly of Span-80, DOTAP, and 1−5% D-$\alpha$-tocopheryl PEG 1000 succinate. SPANosomes exhibited a vesicle size below 50 nm and high siRNA loading capacity, as well as colloidal stability. SPANosomes/siRNA complexes demonstrated efficient silencing activity compared to cationic lipoplexes[202]. A significant finding was that SPANosomes were able to navigate the intracellular environment with superior efficiency, escaping endosomal compartments and ending in the cytoplasm. Microfluidic formulated NSIVs, composed of Tween 85 and cationic charged DDA, show significant efficiency of NISV to transport and transfect GFP siRNA into cells[235].

### *3.1.4. Non-lamellar lyotropic liquid crystalline (LLC) lipid nanoparticles*

Non-lamellar lyotropic liquid crystalline (LLC) lipid nanoparticles, including cubosomes and hexosomes, are in the potential for controlled drug release. Recently, cubosomes have demonstrated promise in drug delivery with unique abilities for

thermodynamic stabilization (Fig. 6A)[205,206,209]. These cubosomes are in bicontinuous cubic structures and feature bilayers curved into gyroid, diamond, or primitive minimal surfaces, creating separate water channel arrays, enhancing colloidal stability and success of RNA delivery[236]. Cuboplexes encapsulating siRNA facilitated the release from endosomes by puncturing pores in endosomal membranes, providing an alternative to traditional proton sponge or electrostatic fusion mechanisms[205,237]. Cuboplexes rely on elasticity energetics to precisely control particle−endosomal interactions, prompting their escape capability due to their unique structures (Fig. 6B)[237].

The incorporation of cationic lipids in cubosome-based systems promotes siRNA encapsulation, stability, cellular uptake, and endosomal escape. Zhen et al.[238] found the superior gene silencing efficiency of nonlamellar DOTAP lipid-functionalized cupoplexes with a low DOTAP percentage (7.5%, *w*/*w*), compared to their lamellar counterpart. Sarkar et al.[206] doped cuboplexes with several cationic lipids and GFP siRNA, achieving successful delivery and gene silencing on GFP-expressing Chinese Hamster Ovary (CHO) cells (Fig. 6D). Introducing small amounts of DOTAP (0.1 mol/mol) has substantially enhanced siRNA encapsulation (75%) without a notable increase in toxicity. The subtle enhancement in transfection efficacy within the first 24 h is attributed to the controlled release kinetics of cubosomes, highlighting their potential for sustained applications. DOTAP modified-cuboplexes demonstrated a 13% superior knockdown efficiency over 72 h compared to commercially available lipofectamine. The subtle enhancement in transfection efficacy over the first 24 h can be attributed to the controlled release kinetics of the cubosomes, highlighting their potential for sustained applications.

The PEGylated cuboplex offers biological stability for RNAi technology by forming a highly ordered internal structure and sterically stabilized design[205]. Encapsulation has achieved a promising gene knockdown efficiency of 75%, exceeding that achieved by traditional LPS-based systems and commercially available siRNA delivery products.

Moreover, conventional cubosome formulations usually possess particle sizes that are too large to meet the requirements for permeabilization of anticancer therapeutics. To address this concern, microfluidic nano-manufacturing approaches were introduced to formulate siRNA cuboplexes with efficient encapsulation and a size of around 75 nm (Fig. 6C)[210].

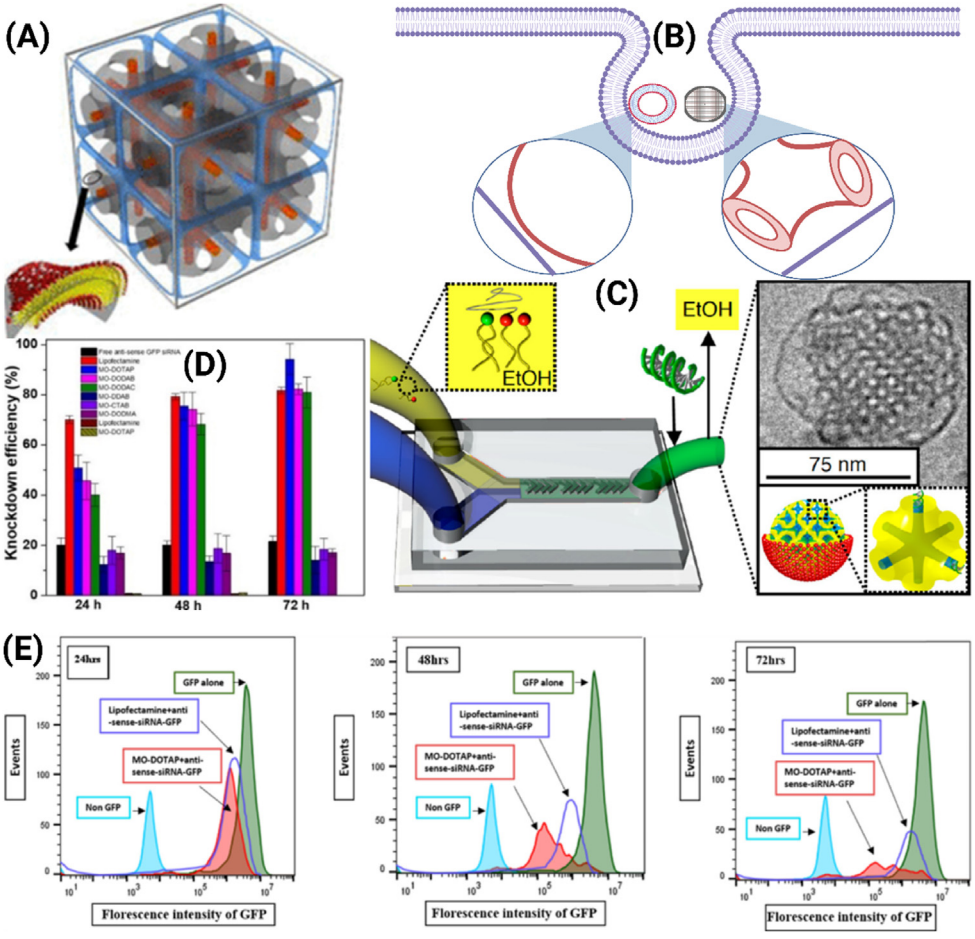


**Figure 6** Cubosomal nanocarriers for enhanced RNA delivery. (A) Bicontinuous cubic Im3m phase structure of cubosomes. Reprinted with permission from Ref. 205. Copyright © 2015 American Chemical Society. (B) Schematic illustration for the cellular entry of lipid nanocarriers. (C) Preparation of cuboplexes by microfluidic staggered herringbone mixer. Reprinted with permission from Ref. 210. Copyright © 2018 American Chemical Society (D) Knockdown efficiency and (E) Fluorescence-activated cell sorting analysis following incubation of GFP-siRNA and different cationic cuboplexes with CHO-GFP cells over 24, 48, and 72 h. Reprinted with permission from Ref. 206. Copyright © 2021 American Chemical Society.

In clinical trials, LNPs continue to represent a beacon of hope and a leading tool in personalized medicine, thanks to their capacity to incorporate and protect nucleic acids while enabling targeted delivery and ultimately mitigating off-target effects. Besides, LNPs have a low incidence of adverse reactions, and they are known to be biocompatible and low immunogenic, making them promising and well-tolerated therapeutic vectors. The diversity of lipids' molecular structure allows for tailored delivery systems that efficiently address specific therapeutic needs in cancer treatment. Despite these strengths, challenges such as low biostability, low cell uptake, immune response modulation, and manufacturing scalability still remain for lipid-based nanosystems. Fabricating LNPs is often based on common bulk mixing or pipette mixing; however, they offer inadequate reproducibility. To address the manufacturing hurdles, microfluidics-based manufacture offers cost-effective, high-throughput, quick, and reproducible LNPs. Microfluidics leverages the principles of laminar flow and precise mixing at the microscale by parallelizing multiple mixing units on a single chip, obviating the demand for complicated fluid handling systems[239,240]. As a first step in converting LNPs into clinically and commercially relevant products, the microfluidic device must be capable of generating high flow rates (>10 L/h)[241]. In the SCALAR platform, 256 mixing units are integrated into a single microfluidic chip, which allows for high-throughput LNP fabrication[242]. Recently, a microfluidic reactor with 128 staggered herringbone mixing channels has been able to generate mRNA/siRNA−LNPs with uniform quality[243]. Specifically, scaling up the microfluidic manufacture of mRNA−LNP vaccines remains challenged by the maintenance of purification and adherence to regulatory compliance with good manufacturing practice (GMP) standards. In addition, maintaining microcahannels sterility presents a further concern for microfluidic-based technologies[244]. Aside from this, ethanol-containing manufacturing protocols require ethanol removal from the final formulation for its potential effect on nanoparticle integrity and encapsulation efficiency[245].

### 3.2. *Polyplexes*

Polyplexes are generated through charge interactions between cationic polymers and negatively charged nucleic acids[246]. Besides being easy to prepare, purify, and chemically tunable, polyplexes ensure that the mRNA is effectively shielded from degradation by RNases through robust protective shields[168]. As well, some polymers allow for efficient cellular uptake and endosomal escape, enhancing transfection efficacy[169]. The merits of polymers extend to distributing biotherapeutics to lymph nodes, promoting robust immune responses[247]. New generations of polymeric nanoparticle delivery approaches, including micelles, polymersomes, nanoparticles, nanocapsules, nanogels, dendrimers, and nanocomposites, offer a promising avenue for the optimal delivery of RNA therapeutics. These nanostructures find applications in regenerative medicine, bioimaging, drug and gene therapy[248]. For instance, polymeric nanogels significantly improve drug loading, stability, and design versatility[249]. Their responsiveness to environmental changes and ability to form complexes with biomacromolecules make them ideal for a wide range of biomedical applications.

Advancements in self-assembling amphiphilic block copolymers have created different nanostructures, such as micelles and polymersomes with a core−shell structure[250]. Polymersomes, composed of amphiphilic triblock copolymers, resemble lipid bilayers and aid the delivery of hydrophilic or hydrophobic molecules. Meanwhile, micelles serve as carriers, usually for hydrophobic agents[251]. Polymersomes, due to their biocompatibility and efficient cellular uptake, surpass micelles in stability and versatility. They encapsulate larger or multiple agents, proving versatile in medical applications.

Synthetic charge-altering polymersomes have been developed to prevent degradation by ribonucleases. They facilitate cytoplasmic protein expression through escaping from endosomes, using the proton sponge effect under acidic endosome environments, guaranteeing structural protection against external threats[172]. A subset of polymeric materials applied in nucleic acid delivery will be discussed accordingly.

#### 3.2.1. *Polyethyleneimine*

Polyethyleneimine (PEI) is well-acknowledged as a non-viral gene transfecting material as it exhibits robust nucleic acid encapsulation and inherent endosomolytic activity at the nanosized level[252]. The structure of PEI inhibits charge repulsion, promoting stability driven by the intrinsic p$K_a$ of the polymer[253]. Linear or branched forms of PEI with high cationic charge density enhance interactions with extracellular matrix components and improve polyplex entry into cells[170]. PEI complex achieves effective endosomal release through the "proton sponge effect", causing osmotic expansion and rupture of endosomes[254,255].

A PEI−mRNA polyplex efficiently transfects multiple sets of cell lines, including bronchial epithelial cells, and nebulization does not adversely affect PEI-mRNA transfection efficiency, so the administration of aerosol is feasible *in vivo*[256,257]. Cortez-Jugo et al.[257] proposed employing the acoustomicrofluidic nebulization technique to facilitate aerosol delivery, demonstrating effective knockdown of lung carcinoma genes. However, linear PEI's cytotoxic effects limit its clinical application[173]. High-molecular-weight PEI possesses notably lower apoptotic and necrotic effects, though it remains detrimental to DNA[258]. Modifications to the PEI structure to generate branches and chemical alterations to enhance biocompatibility paved the way for their therapeutic potential[259,260]. Branched polyethyleneimines (BPEI) possess greater ionization diversity and an improved buffering capacity[261]. Nevertheless, BPEI can form complexes with proteins in the body, resulting in probable immune responses and adverse events[262].

Modifications to BPEI structure, such as incorporating poly(ethylene glycols) (PEGs), generate more biocompatible and biodegradable polymers. These modifications enhance transfection efficiency without affecting PEI−DNA complexation, leading to prolonged circulation time[263]. Further modifications to BPEI, such as conjugation with $\beta$-cyclodextrin or the addition of lipoic acid moiety, have shown promising outcomes in terms of reduced cytotoxicity and improved transfectability[264−266]. Bioreducible disulfides within BPEI structure have shown promising outcomes regarding cytotoxicity and improved transfectability compared to standard BPEI[264].

#### 3.2.2. *Poly(amino acid) derivatives*

Biodegradable polymers derived from amino acids, such as *N*-substituted polyaspartamide (PAsp), have significant biomedical utility due to their good biocompatibility, biodegradability, and safety[267]. Poly[*N*-[*N*-(2-aminoethyl)-2-aminoethyl]aspartamide (PAsp(DET))] has pH-specific protonation, influencing the cationic charge density and facilitating endosomal[268].

Aminoethylene repeats are pivotal in shaping the polymer characteristics affecting cationic charges, endosomolytic, cytotoxicity, and resistance to RNAase (Fig. 7A). The translational efficiency of polyplexes varies with the number of aminoethylene repeats[269].

A further improvement in pH-specific protonation of aminoethylene within the endosomal environment was achieved by incorporating thiourea into the PAsp(TET) structure. Consequently, siRNAs were able to escape and distribute more efficiently within cells as well as exerted better knockdown efficiency (Fig. 7B)[270]. Cationic amphiphilic PAsp(DET) with alicyclic compounds, such as cyclohexyl ethyl, produced stable polyplexes with increased lung-specific mRNA expression. These advancements highlight the potential of poly(amino acid) derivatives for effective nucleic acid delivery[271].

### 3.2.3. *Polyacrylates*

Polyacrylates alone lack effective electrostatic interactions with nucleic acids, and to permit encapsulation, polyacrylate side chain modification is crucial for RNA delivery. At low pH, poly(2-dimethyl aminoethyl acrylate) (PDMAEA) demonstrated their cationic nature, resulting in gradual degradation to innocuous poly(acrylic acid) units[272]. The incorporation of short alkyl methacrylate monomers into the polymeric backbone increased cationic density and improved mRNA entrapment and transfection efficiency with minimal cytotoxicity compared to PEI[273].

PDMAEMA-based cationic star polymers, when coupled with positively ionized dimethacrylate crosslinkers, have been found to effectively transfer siRNA to murine myoblasts that retain lower cytotoxicity[274]. These nanomicelles exhibited a favorable surface charge with low inherent cytotoxicity, efficiently encapsulating and co-delivering siRNA and paclitaxel to cells[275]. Introducing disulfide bonds to the backbone of PDMAEMA consequently created a reductively responsive polymer with minimal cytotoxicity and improved transfectability in melanoma and pancreatic cancer cells[276].

### 3.2.4. *Polyesters*

In an attempt to achieve improved clinical outcomes for polymer-based drug delivery systems, the focus is directed to biodegradable polymers that are less toxic and produce harmless byproducts that can be easily eliminated by renal filtration[278]. Biodegradable polyesters, such as polylactic acid (PLA), polyglycolic acid (PGA), and poly(D,L-lactide-*co*-glycolide) (PLGA), have been broadly investigated for their potential as nanoplatforms for nucleic acid delivery. Lipopolyplexes, formed by modifying polymers with lipids such as DOTAP, DOTMA, and DOPE, improve nucleic acid entrapment and transfection efficiency[279]. Jensen et al.[279] developed an inhalable powder formulation of enhanced green fluorescent protein (eGFP) siRNA using cationic PLGA-based lipopolyplex that shows potential for efficient gene silencing and stability. Pre-functionalization of PLGA or PLA with hydrophilic moieties and/or cationic polymer adjuvants, either PEI, polyarginine, chitosan, poly($\beta$-amino esters) (PBAEs), has been found to enhance nucleic acid encapsulation efficiency and modulate their release[280–283]. PLGA-PEI nanocomplexes coated with hyaluronic acid effectively co-deliver hypericin and HIF-1$\alpha$ siRNA to hypoxic human nasopharyngeal carcinoma cells, demonstrating enhanced gene silencing and tumor accumulation[281].

### 3.2.5. *Charge-altering releasable transporter*

In 2017, the charge-altering releasable transporter (CART) emerged as a promising alternative to traditional cationic polymers[172]. At their core, CARTs are polycations that conveniently encapsulate mRNA due to the positive charges stemming from the oligo($\alpha$-amino ester)[284], are explored to address the rate-limiting mRNA release from internally entrapped cargo for efficient transfection[285]. These carriers deliver polyanionic mRNA under physiological conditions (pH = 7.4) *via* a controlled self-degrading process, transforming into neutral molecules and facilitating the mRNA release from endosomes[172].

However, challenges exist with CARTs, including low transfection efficiency, biodegradability, and cytotoxicity. The limitations can be overcome by modifying lipid and cationic units to create novel CART analogs and incorporating hybrid polyplexes of CART analogs, which showed boosted cellular uptake over standalone CARTs[286]. Waymouth research group reported an amino acid-based CART in 2019, Ser-CARTs, with enhanced biocompatibility and charge-altering properties under different pH

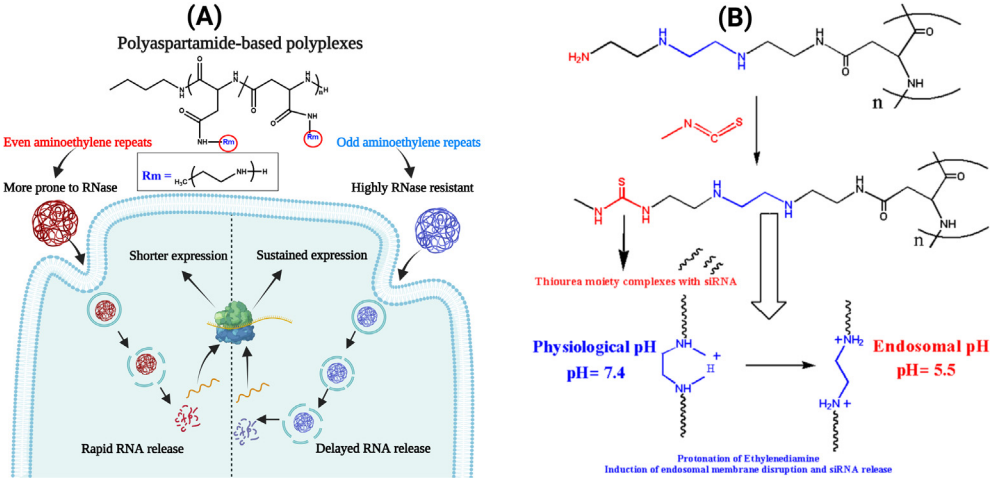


**Figure 7** (A) Classes of *N*-substituted polyaspartamide derivatives and their endocytosis process[277]. (B) Protonation of polyaspartamide derivatives. Figure was made by Biorender.com.

conditions[287]. More recently, lysin-based CARTs were reported for efficient lung-targeted transfection for mRNA and siRNA, demonstrating their effectiveness in cancer vaccination and local immunomodulation in murine models[288]. The administration of Lys-CART/mRNA complex achieved selective protein expression in the lung with no supplementary targeting ligands. The ability to modulate protein expression selectively in the spleen or lung through simple modifications to the CART structure paves the way for innovative biotherapeutics delivery.

### 3.2.6. *Polypeptide and protein-based lipoplexes*

Polypeptide and protein delivery systems are made up of amino acid monomers that confer higher biocompatibility and biodegradability. Common polypeptide carriers include poly L-lysine polymers (PLL)[289], poly oligo-D-arginine[290], and poly L-histidine[291] for delivery of nucleic acids. Like PEI, PLL is cytotoxic to cells with increased cationic charge density, leading to disruption of cell membranes and cytotoxicity. This can be improved by incorporating other monomers, such as carbohydrates, PEGs, and phosphates, into PLL copolymers, reducing the overall charge density and mitigating toxicity concerns[292].

Protamine is a small peptide ~4000 Da, naturally occurring in sperm, often used as an anticoagulant. Protamine has a cationic charge and can form stable nanoparticle aggregates with mRNA, enhancing immune surveillance and cancer cell detection[293]. A protamine-based polylipoplex that incorporates a cationic lipid shell (DOTAP) can further enhance the overall efficiency of mRNA delivery[294]. GALA-complexed mRNA polyplexes, with approximately 350 nm size and slightly negative surface charge, achieved proper transfection in macrophages and DCs.

In the pursuit of overcoming the cellular plasma membrane barrier, cell-penetrating peptides (CPPs) have opened new avenues for the development of effective delivery approaches[295]. The CPPs, including PepFect14 and GALA (glutamic acid-alanine-leucine-alanine repeats), have been instrumental in proceeding with mRNA therapeutic delivery through potential mechanisms of glycosaminoglycan clustering and micropinocytosis[296]. PepFect14 has supported better mRNA delivery both *in vitro* for SKOV-3 cell lines and *in vivo* on the SKOV-3 xenografted mice[297]. GALA offers controlled permeabilization at an acidic pH[298]. GALA-complexed mRNA polyplexes, with a size of approximately 350 nm and a slightly negative surface charge, achieved proper transfection in macrophages and DCs (D1)[299]. GALA polyplexes enter DCs through sialic acid-mediated endo/phagocytosis, leading to an increase in cellular uptake.

### 3.2.7. *Dendrimers*

Dendrimers possess a unique highly branched structure bearing functional surface groups that are useful for drug encapsulation within the spherical structure[300]. Dendrimers, such as polyamidoamine (PAMAM) and poly(propyleneimine) (PPI), have been explored for nucleic acid delivery due to their high cationic charge density and ability to form stable complexes with RNA[301,302]. PAMAMs are effective for RNA delivery hinges on optimizing a set of the dendrimer functionalities, which enhance mRNA binding and contribute to improved stability and transfectability[303].

However, dendrimers may face challenges related to cytotoxicity, immune response, and potential off-target effects, necessitating careful design and optimization for clinical applications. Although efficient in transfection, highly branched PAMAMs are restricted by their notable cytotoxicity due to their increased charged cationic nature[304]. Asymmetric dendrimers composed of lower-generation peptides provide a less toxic alternative, improving cell transfection[305]. There are several approaches currently being considered to improve their biocompatibility, either through disulfide crosslinking[306] or the formation of click chemistry-linked PAMAM-methacrylate polymeric scaffold[307]. Surface modifications with PEG enhance dendrimer biocompatibility, reducing cytotoxicity and immune response. PPI dendrimers modified with PEG and targeting ligands exhibit efficient siRNA delivery to specific cancer cells, resulting in enhanced gene silencing effects[171]. Fluorinated dendrimers have shown enhanced gene transfection efficiency for various genome engineering tools through the improvement of cell uptake, endosomal escape, and serum resistance[308].

### 3.2.8. *Poly beta-amino ester*

The poly(beta-amino ester) (PbAE) was initially developed and demonstrated adequate transfection in human primary cells[309]. The biological attributes of PbAEs render them highly biodegradable and structurally tunable for nucleic acid delivery[310]. Linear PbAE-based delivery systems are constrained by their homogenous backbones. To overcome these limitations, branched cationic polymers, such as highly branched PbAEs (hBPbAEs), were developed for the transfection of nucleic acids and show improved biocompatibility and transfection efficiency compared to linear PbAEs[311].

Since naïve PbAE and nucleic acid complexes are inefficient at delivering mRNA to antigen-presenting cells (APCs), a call for modified PbAE that interact suitably with endosomal membranes, paving the way for cargo release, is necessary[312]. The introduction of poly-caprolactone (PCL) to PbAE has been proven effective in mRNA vaccine delivery for cancer immunotherapy. The success of PCL-grafted PbAEs-based polyplexes led to the development of ionizable polyplexes containing PbAE terpolymer that exhibited a high level of transfection[313].

The research team directed by Stephan has reported that antibody conjugation and polyglutamic acid linkers further improve PbAE-mRNA polyplexes for T-cell targeting, minimizing off-target binding[314,315]. These nanocarriers reprogram circulating T-cells to target the cancerous tissues, avoiding the costly and complicated *ex vivo* T-cell reprogramming process.

Patel and colleagues introduced a method using inhalable BPbAEs-based polyplexes to deliver firefly luciferase mRNA[316]. This approach achieved uniformly distributed mRNA throughout all lung lobes, maintaining stability for up to 90 days. Green and his team reported the delivery of the gene-editing tool CRISPR-Cas9 components with biodegradable PbAE nanoparticles, achieving up to 70% gene knockout efficiency and enabling a 45% gain-of-function expression[317].

### 3.2.9. *Natural polysaccharides and synthetic glycopolymers*

Polysaccharides such as chitosan, hyaluronic acid, dextran, and synthetic glycopolymers offer biocompatible, biodegradable, and less toxic scaffolds for drug and gene delivery[318,319].

Chitosan is a biocompatible, muco-adhesive, and versatile biopolymer that was previously applied for siRNA delivery[320,321]. Modifications of chitosan make it easy to form stable polyplexes with siRNA[322]. Hydrophobic modifications enable encapsulation of hydrophobic drugs within the core and enhance the codelivery capabilities of siRNA complexed in the hydrophilic shell and the hydrophobic drugs[323]. Due to the "proton sponge" effect as an

escape mechanism, conjugation of chitosan with cell-penetrating peptides aids in endosomal RNA escape[324].

Another linear polysaccharide, hyaluronic acid (HA), naturally present in skin and joints, confers its biocompatibility and biodegradability[325]. HA is a primary binding ligand to cluster of differentiation 44 (CD44), a glycoprotein overexpressed in solid tumors. HA-functionalized lipoplexes are attractive for cancer targeting with overexpressed CD44, particularly when paired with cationic lipids[326,327]. Furthermore, cyclodextrins have been found to enhance the delivery of liposomal RNA. A recent study showed that $\beta$-cyclodextrin forms hydrogen bonds with phosphates of siRNA molecules and DOPC LPS, leading to the avoidance of payload escape and improvement in serum stability[328].

To summarize, the polymeric material shields RNA and acts similarly to LNPs in protecting against enzymatic degradation in the extracellular environment, thus prolonging the circulation time in the bloodstream and enhancing its bioavailability. Polymeric nanoparticles may also offer opportunities to customize their physicochemical properties and interactions with biological systems by modulating the composition, size, surface charge, and surface functionalization. Introducing targeting moieties on polymeric nanoparticles' surfaces can optimize their specificity for cell types, minimizing off-target effects. After internalization, nanoparticles must escape lysosomes to avoid degradation and reach the cytoplasm to deliver their RNA payload. The proton sponge effect arising from cationic polymers such as PEI and PAMAM is particularly effective in promoting endosomal escape, leading to osmotic swelling and rupture of endosomes, thereby releasing nanoparticles into the cytoplasm. Moreover, stimuli-responsive polymers have enabled the design of smart nanoparticles sensitive to specific cues within the TME. Anomalous conditions are often observed within the TME, including pH, temperature, redox potential, or enzyme levels. Thus, integrating stimuli-responsive moieties into the polymer matrix allows targeted and controlled release of RNA payloads[329]. Although polymeric nanoparticles show promise for RNA delivery, their clinical benefits are yet to be fully realized. Progress in improving tissue specificity and cellular uptake, biodegradability, biocompatibility and safety, and scaling up the manufacture is essential for clinical translation.

### 3.3. *Exosomes*

Exosomes are a class of extracellular vesicles with a nanoscale size ranging from 30 to 2000 nm. They are originally excreted from mammalian cells. These physiological nanovesicles have gained great attention as potential carriers for pharmaceuticals and biotherapeutics due to their inherent capacity to encapsulate naturally present biological cargo and cross biological barriers. For example, they have been implicated as natural carriers for miRNA[330]. Exosomes show preferential delivery to the brain by crossing the blood−brain barrier and are employed in treating neurodegenerative disorders[331]. Additionally, exosomes could camouflage the phagocytosis and offer a safer platform for biotherapeutics delivery, especially for macromolecules[174]. Zhao et al.[175] developed autologous breast cancer-derived exosomes for cationic bovine serum albumin conjugated siS100A4 for effective lung-targeted delivery. In *in vivo* experiments, loaded exosomes displayed a superior lung affinity compared to LPS, indicating the possibility of integrin-mediated targeting.

Despite the advantages of exosomes like biocompatibility, to date, no FDA approval has been issued for exosomal products[179]. Concerns about how a potentially preloaded cargo may affect patients non-specifically make approval difficult. Exosomes, therefore, require careful production processes to optimize exosome production, purification, and quality control processes to advance their therapeutic applicability[177]. The heterogeneity in exosome size and loaded content poses significant weaknesses, affecting their encapsulation efficiency and yielding inconsistent therapeutic effects[176]. Therefore, adopting GMP for standardized manufacturing processes in clinical trials becomes challenging, given difficulties in characterizing, controlling, and scaling up exosome production through maintaining batch-to-batch consistency[332].

Exosomes can be loaded with desired cargo through various methods, including incubation, transfection-based approach, and physical treatments such as electroporation or sonication[333]. Also, *in situ* assembly and synthesis offer a unique approach, enabling the loading of metal nanoparticles by reducing metal ions within exosomes. Nevertheless, exosomal transfection is highly efficient for small RNAs like siRNA and miRNA, but not for mRNA[334]. The restricted yield of exosomes impedes their use as a therapeutic tool due to limitations on the necessary doses for clinical trials[335].

Scaling up exosome isolation to meet clinical demands presents challenges, given the high costs and limited production quantities. Moreover, the preservation of exosomes for clinical studies remains a major concern, which calls for further research into standard conditions. Current studies recommend storing exosomes at −80 °C, though the storage effects on exosomes depend on their source[336,337]. Storage at this temperature shows concerns about the availability of clinically approved treatments for patients. To address these problems, surface coating of exosomes shows promise to preserve longer shelf life, such as the nanofilm of supramolecular complexes of ferric ions and tannic acid[338] or PEG modification[339]. These methods enhance stability at specific temperatures and biocompatibility, potentially advancing exosome-based nanoplatforms for more effective, safe, and stable therapeutic applications.

In closing, researchers are exploring the potential of these natural nanovesicles for RNA delivery due to their biocompatibility, minimal immunogenicity, and ability to permeate biological barriers. Another advantage of exosomes lies in their ability to display target-specific surface ligands, enabling them to precisely target specific cell types and tissues. Various loading methods have been implemented to successfully incorporate RNA into exosomes, including electroporation, incubation, sonication, and transfection with lipid-coated particles. Despite the potential of exosomes for RNA delivery, optimizing exosome manufacturing, purification, and loading remains challenging. Addressing the concerns associated with exosome yield, cargo loading, and tissue tropism would help advance exosomes into the clinical phase[178]. Therefore, exploring innovative approaches, such as *in vivo* engineering of cells, 3D bioprinting, and microfluidics to produce exosomes for therapeutic purposes will pave the way to eliminate the cost and scalability challenges associated with exosome isolation[340].

### 3.4. Organic-inorganic mesoporous silica nanohybrids

Standard mesoporous silica nanoparticles (MSNs), such as MCM-41 and SBA-15, are widely known for their significance in nanomedicine owing to their remarkable surface area and pore volume. MSNs have the potential for dual delivery of anticancer therapeutics, doxorubicin, and siRNA targeting P-glycoprotein through surface decoration with a PEI-PEG copolymer. This modification facilitated the protected delivery of both agents with improved EPR with reduced uptake in xenografted nude mice and led to synergistic inhibition of tumor growth[181]. The application of MSNs is restricted to small drug molecules due to nanopore size <5 nm[341,342]. Large open pore MSNs with a pore diameter of >12 nm present a prospective siRNA delivery approach[181−183]. Ultra-large MSNs protect loaded RNA from degradation and significantly enhance the transfection efficiency, cytotoxicity, and gene silencing *in vitro* and *in vivo* (Fig. 8)[182].

Wang et al.[343] generated dendritic mesoporous organosilica (DMSONs) with a small diameter (∼50 nm) and large pore size (>20 nm). The DMSONs exhibited significantly improved *in vitro* mRNA transfection efficiency. The same group further studied mRNA delivery by combining DMSONs with zeolitic imidazolate framework-8[344]. In this system, Zeolite adds positive charges and large mesopores that are appealing for effective mRNA loading, and its imidazole ring eases the endosomal escape (Fig. 9). These merged effects culminated in enhanced mRNA translation and improved transfection efficiency, surpassing the performance of commercial products.

Hollow MSNs (HMSNs) with larger pores having diameter of ∼24 nm allowed the encapsulation and controlled release of siRNA targeting P-glycoprotein and doxorubicin in a manner that reduced the tumor volume by 5.93-fold less than untreated doxorubicin [342]. The increased efficiency of the doxorubicin is attributed to the synergistic impediment of multi-drug resistance in subcutaneous xenografted mice.

Xiong et al.[183] synthesized a novel magnetic core−shell MSN with a particle size of ∼150 nm and radial mesopores of 12 nm, showing high siRNA loading (2%, *w/w*) and responsiveness to an external magnetic field. Although the siRNA-loaded MSNs showed a poor surface charge (5.4 mV), this problem was resolved by clogging the silica pores with a pH-responsive gatekeeper (tannic acid). Tannic acid is a dual role player in safeguarding the loaded siRNA cargo, enhancing the surface charge (−53.1 mV) and dispersion stability. With the assistance of the external magnetic field, the functional siRNA uptake into the cytoplasm of human osteosarcoma cancer cells was notably improved to 40% within 6 h. Furthermore, bowl-like MSNs with non-aggregated structures of ∼180 nm and cavities of ∼140 nm, along with amine functionalization, are suitable for pDNA delivery[345]. The loading efficiency of the nano-bowls was improved due to the accessibility of its crescent-shaped central cavity, which is absent in previously discussed silica-based nanoparticles.

### 3.5. Carbon-based nanostructures

A growing area of research for RNA delivery is carbon-based nanomaterials, thanks to their unique structure and electrical, thermal, optical, and chemo-mechanical properties[186]. The application of carbon-based nanomaterials in RNA delivery encompasses a wide range of carbon allotropes, such as graphite, fullerenes, nanotubes, and diamonds with diverse nanostructures. Carbonaceous surfaces can be modified to bind with RNAs, address poor water solubility, and avoid toxicity and structural problems.

Fullerenes belong to a particular category of carbon allotropes, capable of forming stable complexes with nucleic acids and carrying RNA cargo, facilitating cellular uptake and endosomal release. Tetra(piperazino)[60]fullerene epoxide (TPFE) is a cationic fullerene that was developed by the Nakamura group at the University of Tokyo for effective inhalable delivery of siRNA (Fig. 10A)[346]. Molecular dynamics simulations showed that this cationic fullerene has a strong tendency to embed into siRNA's major groove, forming a stable binding pattern with a high affinity for siRNA. Thus, fullerene guarantees stability against nucleases and pulmonary surfactants with comparatively higher RNAi activity (Fig. 10B).

Carbon nanodot is a zero-dimensional form having a size of ∼10 nm and shows the potential to deliver siRNA when decorated with polycations[348]. Carbon dots may pose potential safety concerns. However, the passivation of nanodots with polycations such as PEI, alkyl PEI, PLL, and PAMAM has enhanced biocompatibility and transfectability[349]. PEI-modified nanodots and HER3 siRNA combined with HER2 targeting drug (trastuzumab) have improved their efficiency against breast cancer[350]. The amphiphilic characteristics of carbon dots facilitate their binding with RNA, and their fluorescent properties allow tracking the delivery process. The developed carbon nanodot, O12-Tta-CDs, have exhibited optimal OVA-mRNA transfection efficiency and can target the spleen for improved antigen presentation and T-cell infiltration (Fig. 11)[351].

Similarly, graphene nanodots can be conjugated to PEI for mRNA delivery. Graphene quantum dots demonstrate better biocompatibility than carbon allotrope due to residual metal impurities. Liu et al.[187] investigated the PEI-modified graphene/mRNA nanocomplex through electrostatic interactions, forming nanoparticles with sizes below 300 nm. These dots could deliver intact eGFP mRNA to hepatocarcinoma cells with dose-dependent expression, but the need for higher doses caused safety concerns that should be addressed.

Carbon nanotubes, including single-walled (SWCNTs) and multi-walled (MWCNTs), are potential materials for siRNA delivery. Due to the high aspect ratio of SWCNTs, they offer a promising nanoplatform for loading RNAs with high capability[188]. Despite the potential endocytosis-independent cytoplasmic entry, CNT-PEI and CNT-pyridinium exhibited only modest silencing activity accompanied by moderate to high cytotoxicity[189]. In 2017, Liu et al.[347] applied modified SWCNTs with PEG-phospholipids to deliver siRNA (Fig. 10C). This modification effectively transported and released CXCR4 siRNA into human T-cells and peripheral blood mononuclear cells, exhibiting superiority compared to conventional liposomal formulation (Fig. 10D). SWCNT complexes demonstrated around 55% knockdown of CXCR4 receptors on T-cells within 24 h, which increased up to ≈90% by the third day.

Siu et al.[352] have functionalized SWCNT with succinated PEI to make it water-soluble to deliver Cyanine3-labeled Braf-specific siRNA. These complexes exhibited sound gene silencing *in vitro* in B16−F10 cells. At the same time, *in vivo*, a significant siRNA uptake in a C57BL/6 mice melanoma model was observed over the course of 25 days through topical application, demonstrating a substantial decline in tumor growth. The paramagnetic MWCNTs were reported to disrupt cell membrane integrity due to self-rotating under a weak rotating magnetic field, suggesting these MWCNTs can be used for nucleic acid delivery through the non-

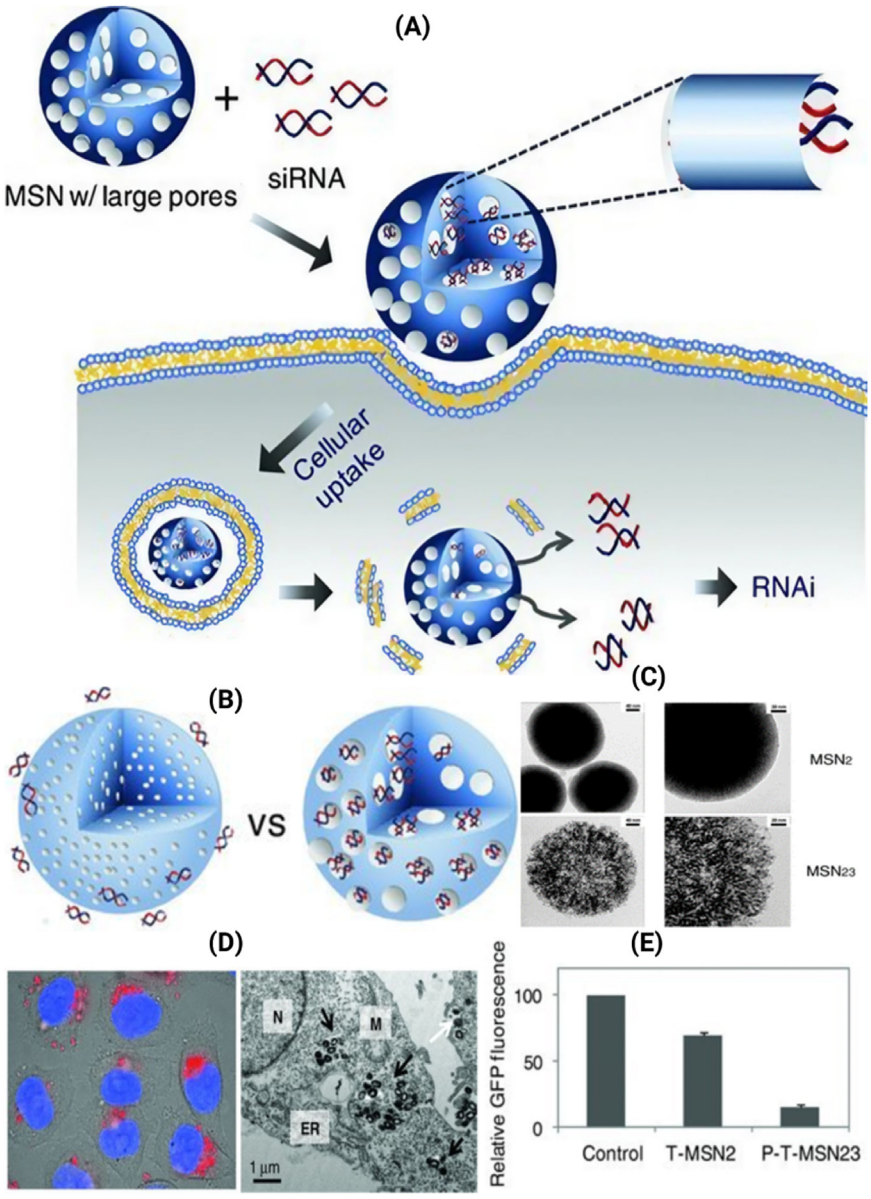


**Figure 8** Ultra-large mesoporous silica for enhanced siRNA delivery. (A) Delivery of GFP siRNA using ultra-large pore MSNs. (B) siRNA loading to small pore and ultra-large pore MSNs. TEM images of (C) small pore (MSN2) and Ultra-large pore MSNs (MSN23). (D) Observation of MSNs after cellular uptake. (E) Relative GFP expression analysis showing that large pore MSNs (MSN23) were the most effective in promoting gene delivery in GFP-expressing Hela cells. Reprinted with permission from Ref. 182. Copyright © 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

lethal magnetoporation[190]. However, magnetoporation can compromise cell viability, leading to leakage of cell components and magnetolysis, which can be beneficial for *in situ* tumor ablation. Both processes can be obtained but under different levels of controlled magnetic field flux density.

Nanodiamonds with $sp^3$ carbon atoms and diameters $\sim$ 20 nm are options for RNA delivery, with coatings for specific targets. Nanodiamonds can be coated with non-covalent cationic polymers or covalent interaction such as sialylation with a coupling agent ending with methacrylate that can further link to cationic polymers (Fig. 12)[353,354]. Despite its successful use in inhibiting Ewing sarcomas and breast cancers[355,356], challenges like complex functionalization processes and biodegradability consequences still require attention. Nanodiamonds have been applied to deliver siRNA targeting the Spalt-like transcription factor 4, abundant in hepatocellular carcinoma, and exhibited increased cellular uptake compared to Lipofectamine 3000 at a weight ratio of 50:1[357]. These complexes have shown remarkable knockdown efficiency in SNU398 spheroids ($P < 0.01$) and a significant decline in AKT, phosphorylated AKT, and Cyclin D2 expression downstream.

### 3.6. *Metallic nanoparticles*

The versatility of gold nanoparticles is reflected in their ability to be chemically resistant, enzymatically stable, less cytotoxic, and physiochemically tunable, along with the optical properties that render them valuable for photothermal therapy[192]. Thiol-based interactions enable siRNA to bind to gold nanoparticles for

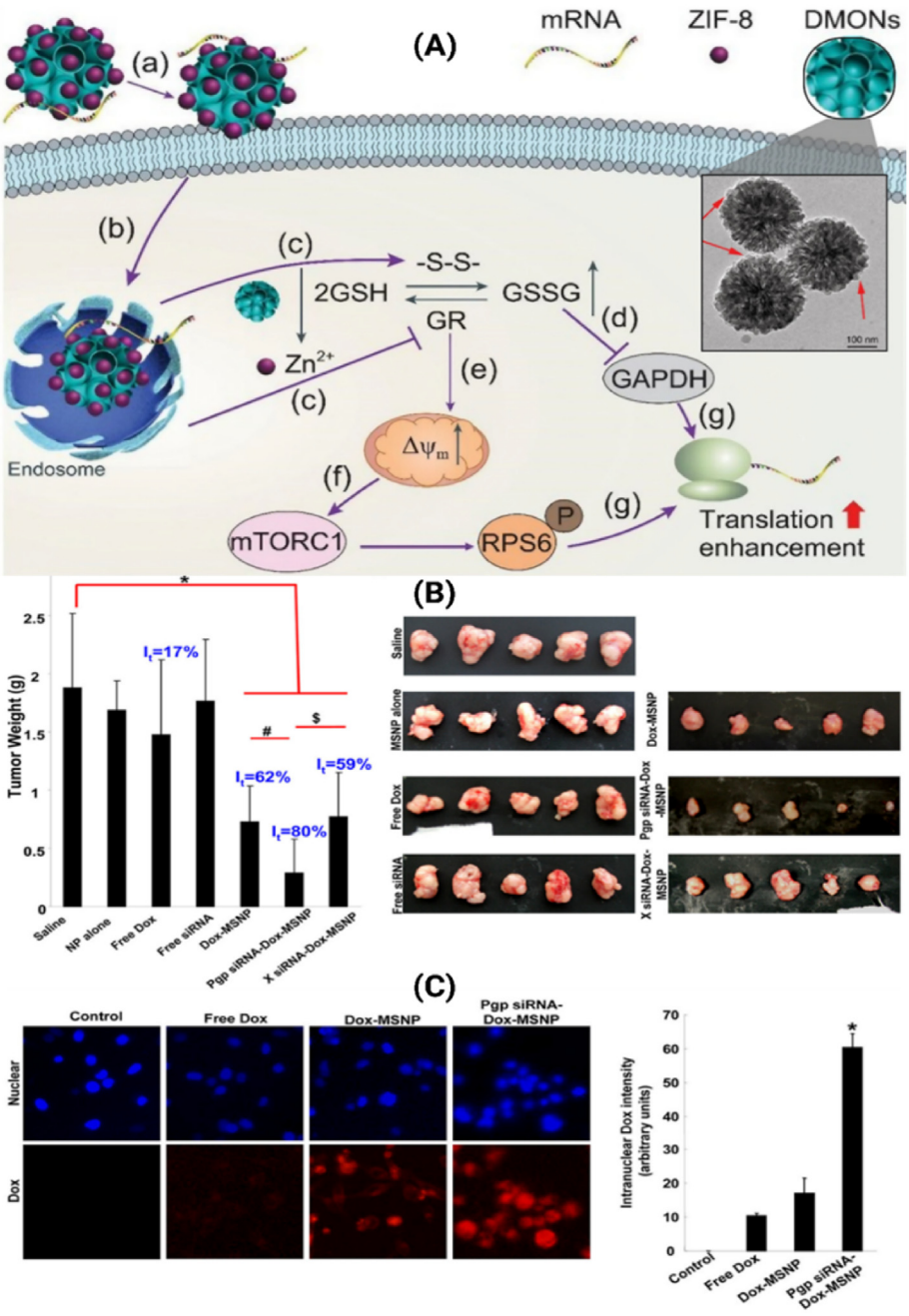


**Figure 9** (A) Improvement of mRNA expression *via* DMSONs. Reproduced under Creative Commons from Ref. 181. Copyright © 2020, The Author(s) 2020. (B) Assessment of antitumor effect for Dox-loaded MSNP with and without P-glycoprotein siRNA versus controls. (C) The improved flux of Doxorubicin in MCF-7/MDR cells is presented in fluorescent images. Reprinted with permission from Ref. 344. Copyright © 2013, American Chemical Society.

enhanced cellular delivery. Further coated with PBAE, it allows multifunctional designs with adhesion and penetration-enhancing peptides[358]. Xue et al.[193] assembled gold nanoparticles with Y-shaped backbone-rigid triangle DNA bricks with sticky ends for siRNA delivery. This forms a protective covering layer surrounding siRNA. The release of siRNAs was responsive to cancer-specific endogenous miRNA, enabling targeted gene silencing within cancer cells with impressive *in vivo* therapeutic efficacy, showing complete inhibition of tumor growth.

Polymer-coated superparamagnetic iron oxide nanoparticles (SPIONs) are well suited for targeted delivery of nucleic acids by leveraging their unique size and superparamagnetic properties. SPIONs can dually target the payload, employing ligands and magnetic guidance. A PEI-SPION formulation has been used for the delivery of ADAM10 siRNA to treat prostate cancer[194]. In this formulation, the nanocomplex was conjugated to PEG to enhance the biocompatibility of the nanocomplex. The cellular uptake of SPIONs was evident by the confocal microscope, which had particle sizes of ~20 nm as detected by TEM.

In magnetofection, the magnetic nanomaterials are complexed with vectors to help them to be in proximity with target tissues, enhancing their uptake *via* endocytosis[359]. This approach involves

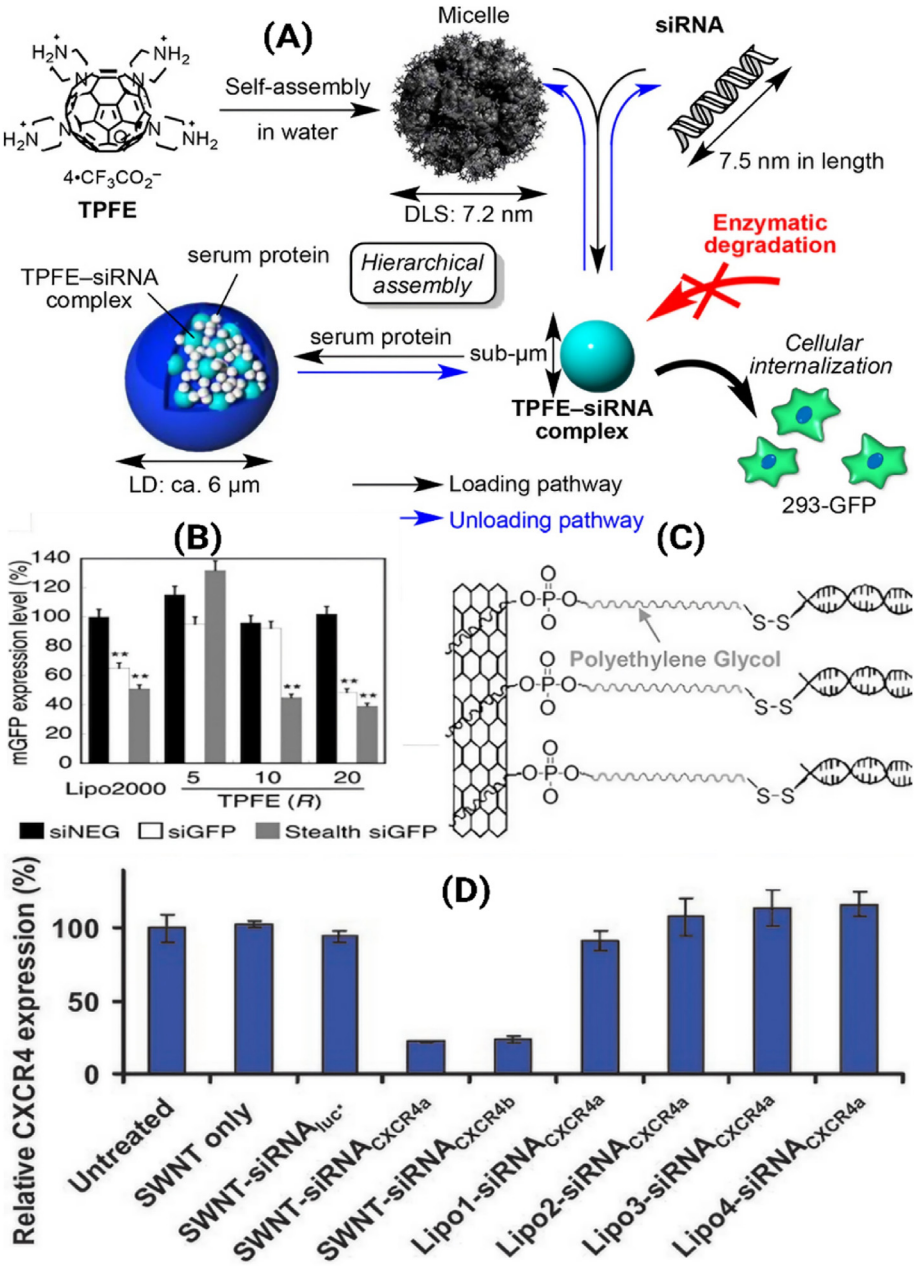


**Figure 10** (A) Presentation of TPFE self-assembly. (B) TPFE-based siRNA RNAi efficiency *in vitro* using an eGFP non-specific siRNA (siNEG) and two eGFP-specific (siGFP and Stealth siGFP) at different TPFE/siRNA ratios (R: 5 to 20). Lipofectamine 2000 (Lipo2000) serves as a standard transfection agent. (**$P < 0.01$ *vs.* siNEG). Reprinted with permission from Ref. 346. Copyright © 2018 American Chemical Society. (C) SWCNTs surface decoration with $NH_2$-terminated PEG-lipid for disulfide linkage with siRNA. (D) Assessment of CXCR4 expression on CEM cells post-treatment, including liposomes (Lipo1−4) and luciferase (Luc) or siRNA control. Reprinted with permission from Ref. 347. Copyright © 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

gene transfer using SPIONs coated with nucleic acids in the presence of a magnetic field [195]. Nevertheless, there are persisting difficulties ahead of *in vivo* applications of magnetofection, including lower transfection efficiency, particle size requisites, rapid systemic clearance, and safety concerns with repeated administration[196].

## 4. Challenges and barriers in RNA delivery to tumors

As much as mRNA and RNAi technologies have appealed to Biotech companies, there are still several unresolved challenges pertaining to their development. The challenges of RNA delivery include overcoming barriers related to stability, cellular uptake, immune response, and targeted delivery. RNA molecules are rapidly degradable by RNases and nucleases; therefore, they need to be incorporated into formulations[360]. Efficient cellular uptake is crucial for the therapeutic efficacy of RNA, and addressing endosomal escape is a common obstacle[361]. Immune responses triggered by RNA can lead to unwanted reactions, requiring strategies to mitigate immunogenicity[34,362]. Finally, achieving targeted delivery to specific tissues or cells while minimizing non-specific organ deposition and off-target effects poses a significant

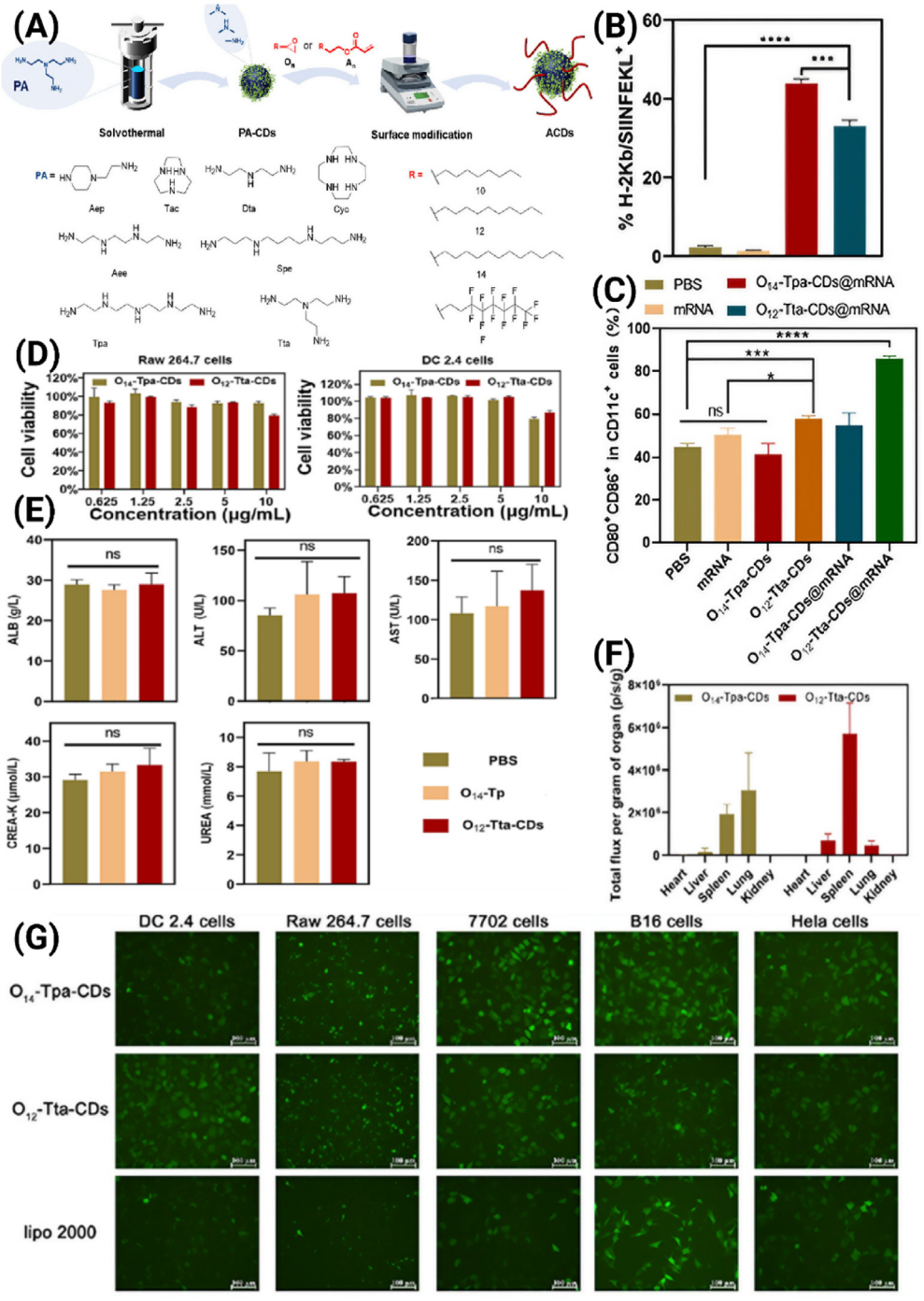


**Figure 11** Amphiphilic carbon dots (ACDs)-based delivery system. (A) Preparation of ACDs. (B) Percentage of maturated antigen (H-2Kb/SIINFEK$^{+}$) presenting cells, and (C) Proliferation of CD80$^{+}$ and CD86$^{+}$ cells in bone marrow DCs following treatment of bone marrow DCs by ACDs. (D) Cell viability assessment of ACDs-treated DC2.4 and Raw264.7 cells, showing biocompatibility. (E) Measurement of serum toxicity markers (serum albumin, ALT, AST, Creatinine, and urea) following ACDs administration at doses of 3.6 mg/kg to mice. (F) Flux analysis of ACD flux in mice's excised organs. (G) Fluorescence of expressed GFP after incubation with ACDs compared to LPS. Reprinted with permission from Ref. 351. Copyright © 2023 American Chemical Society.

challenge in RNA delivery technologies[363,364]. Furthermore, the co-delivery of gene therapies and chemotherapies requires a nanocarrier that maintains a synergistic effect with minimal side effects[365]. In the long run, once preclinical studies have been completed and all obstacles have been handled, gene therapy will have to be approved by regulatory agencies to ensure the product is effective, reproducible, and scalable[366]. Stability issues, which often require demanding storage and stringent handling measures, could compromise the dispatch and distribution of RNA therapeutics.

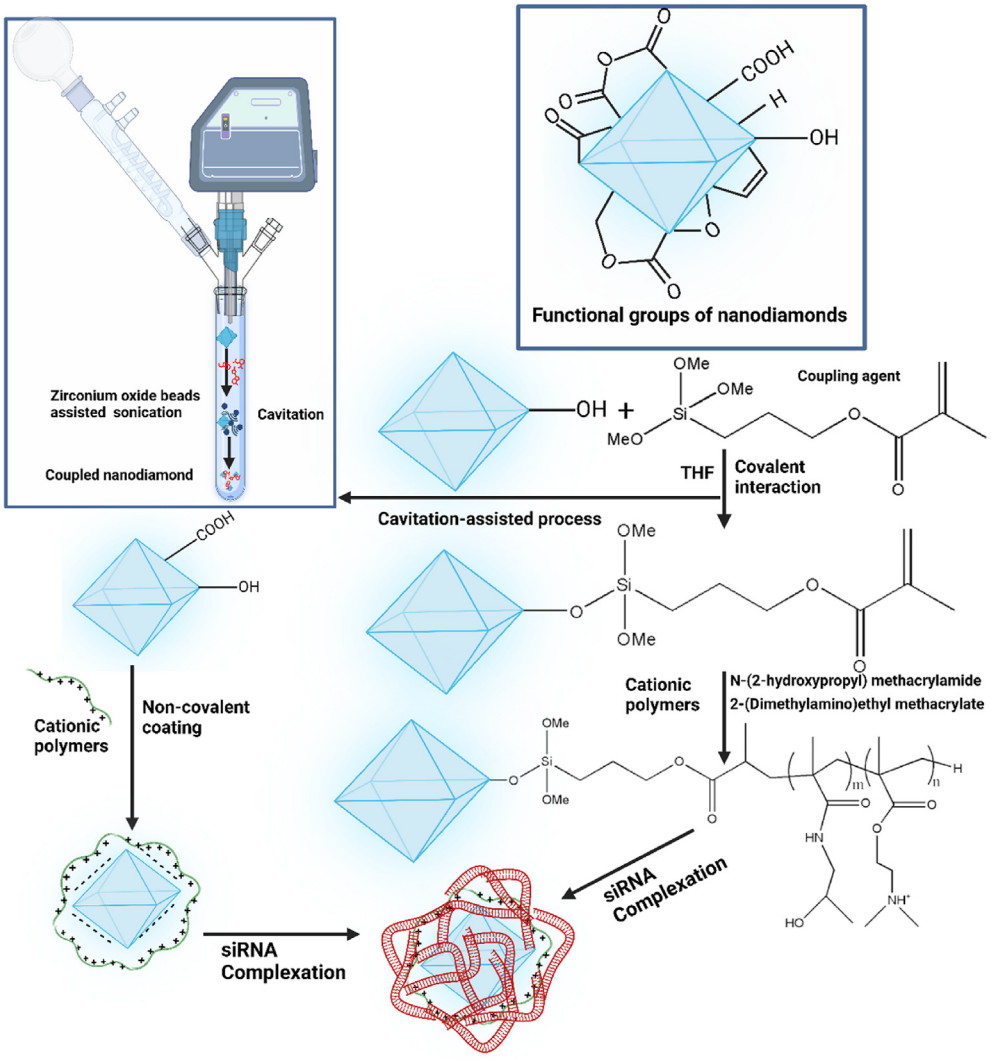


**Figure 12** Nanodiamonds for RNA delivery. Nanodiamonds can bind to siRNA using two methods: a covalent interaction and a non-covalent binding approach. The covalent interaction involves surface modification with sialylation through cavitation-assisted processing with zirconium oxide beads, followed by coupling with methacrylamide and a cationic polymer to form a complex with siRNA. In the non-covalent method, nanodiamonds are coated through electrostatic interaction with cationic polymers, which are then complexed with siRNA. Figure was made by Biorender.com.

### *4.1. TME-associated barriers to RNA delivery*

The path lies ahead for RNA-based therapeutics before they can effectively reach their intended target within the intricate TME. Abnormal vascular networks, influenced by numerous essential components within the TME[367], pose a major concern to their transport and release into target cells. The influential factors governing the eventual *in vivo* fate of RNA therapeutics are primarily TME, extracellular matrix (ECM), and increased interstitial fluid pressure (IFP).

Tumor hypoxia, arising from abnormal blood vessels, increased metabolic rate, dense and fibrotic TME, genetic mutations, and increased tumor size, creates barriers to oxygen diffusion and poses challenges for delivering therapeutic RNA molecules to target tissues[368]. Chronic hypoxia, marked by the formation of a necrotic core, further complicates the situation[369].

Under normoxia, the constant degradation of the alpha subunit of hypoxia-inducible factor 1 (HIF-1) complex is mediated by dioxygen-dependent mechanisms[370]. However, under hypoxia, HIF-1$\alpha$ stabilizes, leading to metabolic reprogramming in cancer cells and impacting various cellular processes[371]. The alpha subunit of HIF heterodimer complexes with the beta subunit, which is then translocated to the nucleus, where it triggers hypoxia response elements (HREs) on specific gene promoters, initiating transcription of HIF-1 genes and subsequent cellular adaptation to hypoxia (Fig. 13)[372]. HIF-1 facilitates vasodilation by stimulating inducible nitric oxide synthase (iNOS), increasing oxygen supply to the tumor. This positive HIF-1$\alpha$−iNOS feedback loop influences the TME, and the ensuing hypoxia induces changes in cancer cell metabolism, including increased reliance on glycolysis and reductive glutamine flux.

Further changes to the TME occur as a counteracting response to tissue ischemia. HIF facilitates vasodilation by stimulating inducible nitric oxide synthase (iNOS), increasing oxygen supply to the tumor[373]. The positive HIF-1$\alpha$-iNOS feedback loop affects the TME[374], and the resultant hypoxia induces changes in cancer

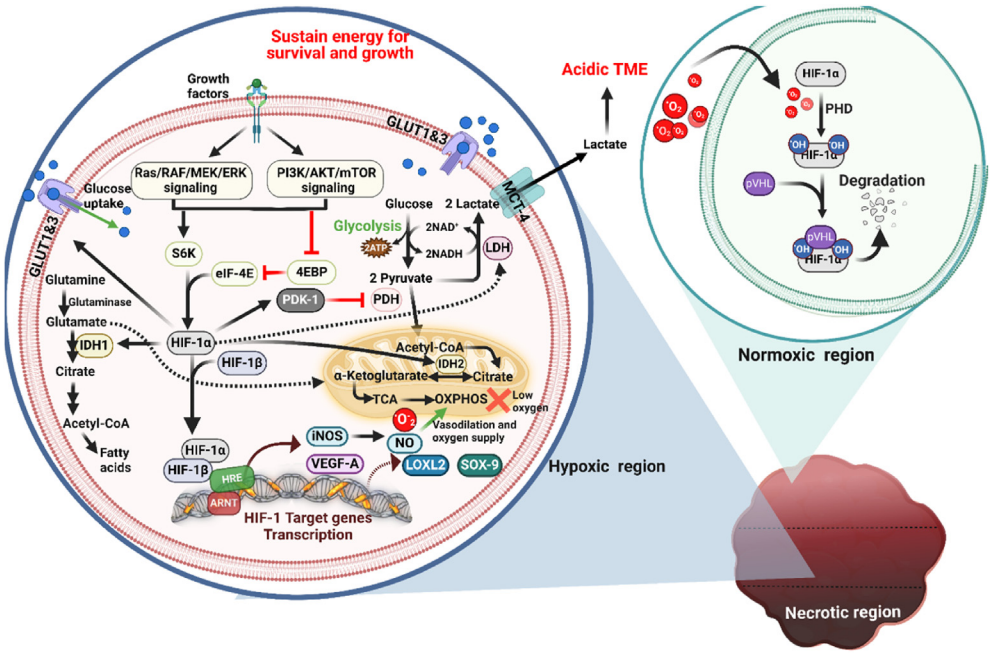


**Figure 13** Effects of HIF-1 on the cellular adaptation mechanisms to hypoxia. HIF-1 is a major contributor to changes in TME as it plays a crucial role in adapting to tumor-associated hypoxia through the expression of VEGF-A, regulating angiogenesis, and improving oxygen supply *via* vasodilation triggered by iNOS. Furthermore, HIF-1 enhances glucose uptake and glycolysis, relying on the anaerobic pathway for pyruvate metabolism over the TCA pathway to generate sufficient energy under low oxygen conditions. The regulation of LOXL2 and SOX-9 expression is mediated by HIF-1, contributing to ECM stiffness and thereby hindering nanoparticle delivery. Figure was made by Biorender.com.

cell metabolism[375], including increased reliance on glycolysis[376] and reductive glutamine flux[377].

Hypoxia also increases the stiffness of ECM within TME. The ECM comprises various proteins (*e.g.*, fibronectin and collagens), forming a dense matrix that creates a physical barrier to nanomedicine diffusion to the tumor core, such as nanoparticles or anticancer drugs (Fig. 14)[378]. Moreover, the negatively charged glycosaminoglycans in the ECM impede the deliverability of nanocomplexes-carrying nucleic acids, limiting the effective immunotherapy[379]. The gradient of escalating stiffness of ECM effectively steers T-cells and DCs away from direct interaction with the tumor cells and accumulates in the stroma, preventing them from reaching the tumor cells.

Within TME, the ECM influences the polarization of tumor-associated macrophages (TAMs) to the M2, leading to altered pro-inflammatory or anti-inflammatory responses[380]. M2 macrophages also contribute to the suppression of immune responses against tumors by impeding CD8+ T-cell proliferation, including the recruitment of Tregs *via* CCL22. They secrete inhibitory IL-10 and TGF-$\beta$, as well as iNOS-mediated free radical generation, etc[381].

Cancer-associated fibroblasts (CAFs) remodeled the ECM, resulting in high heterogeneity and lack of specific markers and creating challenges for identification. CAFs also cause the failure of immunotherapeutics by controlling matrix protein production and leukocyte infiltration[382,383]. ECM primary components such as collagens I, II, XVII, and elastin can trigger immune inhibitory signaling in NK cells, converting it to tissue-resident NK and limiting its cytotoxicity[384,385]. As well, ECM and the associated proteins exert other effects on TME through the regulatory rules in the escape mechanisms, including the expression of checkpoint molecules[386,387]. The impact of ECM components on immunotherapies has led to the emergence of strategies to address ECM-associated barriers, including enzymatic degradation of ECM components or co-delivery of drugs with ECM-modifying agents[388].

As the tumor grows and exceeds the size of 2−3 $mm^3$, angiogenesis occurs when oxygen and nutrients fail to meet the cells' needs to survive[370]. As a result, the formation of new blood vessels leads to the EPR[389]. However, the advantage of EPR in delivering nanoparticles to the tumor site is counteracted by increased IFP (Fig. 14)[390]. The increment of IFP in the tumor region impedes the penetration of therapeutic nanoparticles into the tumor mass[391]. An organized blood supply within more advanced tumors allows for depositing therapeutic nanoparticles, although often limited to peripheral areas. The augmented IFP impedes the delivery of anticancer therapies, particularly to the core of the tumor, reducing treatment efficiency and leaving residual cells to initiate an environment, thus increasing the risk of tumor recurrence. A study has reported the positive effect of methyl-selenocysteine on angiogenesis and normalization of the vasculature, contributing to augmented tumor delivery[392]. Methyl-selenocysteine acts by decreasing vascular leakiness while reducing microvessel density and increasing pericyte coverage. Therefore, vascular normalization mitigates the increased IFP, allowing drugs to be delivered and absorbed more efficiently within the tumor.

A thorough understanding of tumor vascular density, angiogenic factors, and nanomedicine delivery mechanisms is key to advancing more effective approaches to tumor targeting. Miscellaneous nanoplatforms harness the EPR effect to deliver drugs and genes. Besides EPR and IFP, there are other contributing factors that affect tumor uptake and accumulation, including vascular density and tumor doubling time[393,394]. In TME, there is a

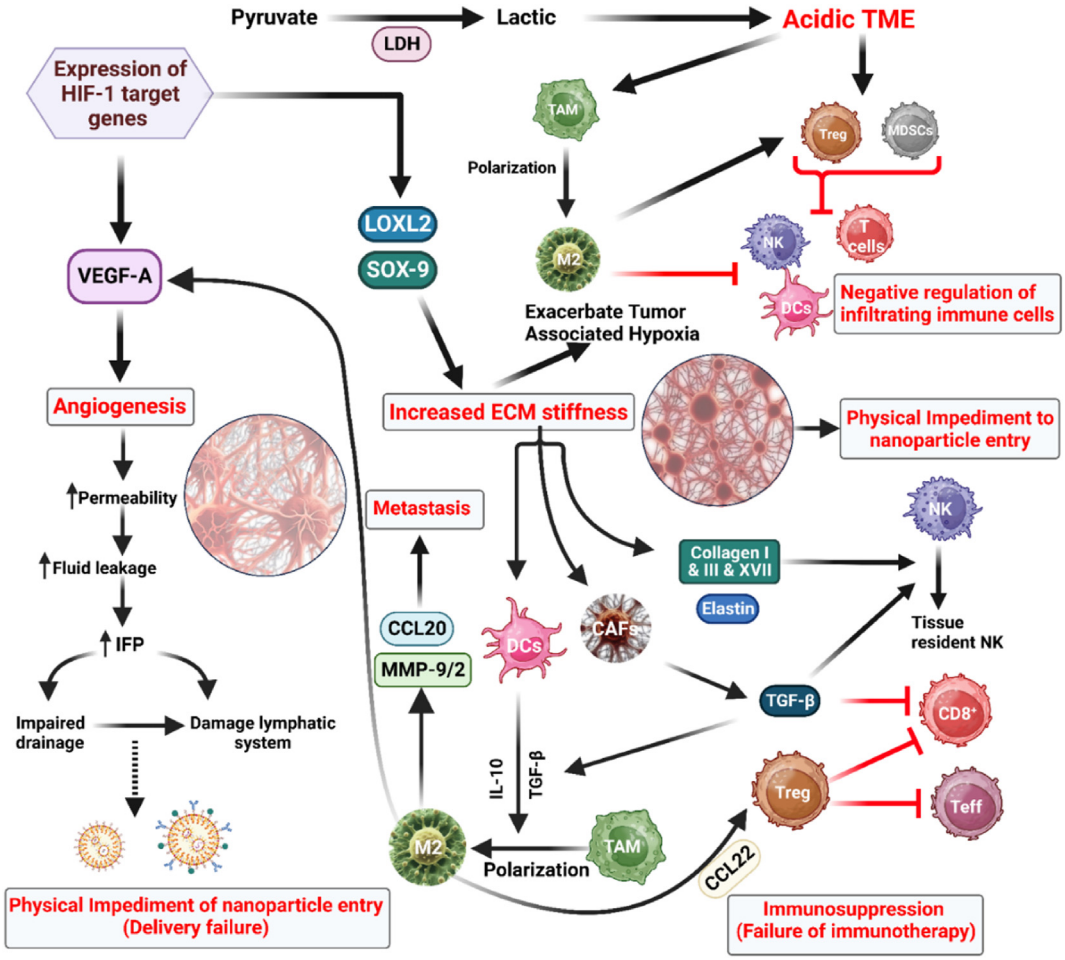


**Figure 14** TME-associated barriers to immunotherapy delivery and action. Resistance to cancer immunotherapeutic formulations arises primarily from heightened IFP and the immunosuppressive nature of TME. Elevated IFP, a consequence of VEGF overexpression, leads to hyperpermeability, impairing lymphatic tissue drainage and posing obstacles for nanoparticle mobility. Conversely, components of the stiff ECM prompt cancer-associated fibroblasts (CAFs) and DCs to collaboratively hinder the function of cytotoxic immune cells, such as Natural Killer cells (NKs) and $CD8^+$. Additionally, this environment promotes cancer development and metastasis through the expression of immunoinhibitory cytokines (IL-10 and TGF-$\beta$). Figure was made by Biorender.com.

noticeable difference in blood vessel density between tumor types and stages, which, in turn, influences tumor accumulation and treatment efficiency. For example, hepatic and renal carcinoma is characterized by its highly vascularized blood networks, which can facilitate tumor accumulation of therapeutics[393]. An analysis spanning ten years of nanoparticle delivery has shown that skin, pancreatic, brain, and liver cancer had a relatively higher accumulation of around 1.3%, 0.8%, 0.8%, and 0.7% of the total injected dose compared to other tumor types[394]. However, these dense networks may accelerate the progression to the metastatic state[395]. Meanwhile, ovarian carcinoma may manifest relatively less dense microvascular networks, compromising therapeutic agent accessibility[393,394].

Furthermore, researchers developing EPR-based nanoplatforms for tumor treatment should consider tumor doubling time (TDT) variance when designing gene therapy release rate for uptake by malignant tissue[393]. TDT varies based on the type, grade, and metastatic status of the tumor and can range from a few days to as long as a year in the case of pituitary adenomas, leading to heterogeneous outcomes[396]. Fast-releasing formulations may be necessary for treating quickly multiplying tumors[393]. With each tumor doubling cycle, the amount of drug required to kill a given number of cells doubles, raising concerns about the safety of the RNA-based drug or vector. In other scenarios, sustained release and uptake formulations are preferable for tumors with long TDT.

### 4.2. *Macromolecule-related challenges*

#### 4.2.1. *Size and charge*

The primary challenges in the delivery of RNA-based therapies stem from the large size and high charges of these macromolecules, which limits their bioavailability. This was particularly observed with mRNA vaccines due to their negative charges and large size (up to 5000 kDa), while the RNAi tools are usually around 4−15 kDa[32]. The increased size and charge present obstacles to systemic delivery, requiring the use of cationic, ionizable lipid, or polymeric nanoparticles[397]. Unlike ASO, which attaches to the mRNA target in an unaided fashion, other RNAi tools undergo several processing steps prior to loading into catalytic RISC/Ago2 complex and exerting silencing effects (Fig. 2). Therefore, siRNAs have specific biological prerequisites for maintaining activity. Several attempts have been unsuccessful in

enhancing their delivery through chemical modification or conjugation to other molecules. This requires careful replication of the A-form structure, minor groove contacts, and the charged phosphodiester backbone. Recent advancements in siRNA structural manipulation technologies aimed to enhance *in vivo* performance; the size and charged nature of this macromolecule dictate the presence of a carrier to work efficiently.

#### 4.2.2. *Stability*

Achieving the desired clinical outcomes for RNA therapeutics relies on their stability *in vitro* and under physiological conditions until they are delivered to the target. RNA displays greater susceptibility to oxidation *in vitro* compared to DNA[398]. Meanwhile, *in vivo*, RNA undergoes enzymatic degradation primarily mediated by RNases, endonucleases, and 5′ exonucleases[360]. Therefore, it is of utmost importance to ensure RNase-free conditions throughout the entire RNA preparation process, storage, handling, and administration. Similarly, the relatively short phosphodiester backbones of ASOs make them vulnerable to nuclease degradation because of their highly charged and hydrophilic characteristics. Nevertheless, second-generation ASOs showed superior resistance to nucleases, exhibiting improved stability and a longer half-life[399].

Several strategies have emerged to protect RNA molecules; one general approach entails introducing them into lipid or polymeric nanoparticles to mitigate their sensitivity to RNases. Despite that, new hurdles have emerged since the RNA molecules are subjected to hydrolysis during preparation and storage within the hydrophilic core of the lipid nanovesicles[400]. Additionally, lyophilization was undertaken to minimize the effects of hydrolysis in the liquid form. However, RNA stability can still be potentially damaged by freezing and thawing cycles, which necessitates the addition of cryoprotectants[401]. Further approaches that hinge on modifying nucleosides or optimizing the sequence are often adopted to increase the inherent stability of RNA molecules[402].

#### 4.2.3. *Endosomal escape*

There should be careful engineering of RNA vectors to bypass the internal biological barriers, such as the epithelium and endothelium, that hinder RNA transfer from the blood to organs[403]. Immediately after RNA-nanoplatforms are taken up by cells, endosomes are generated. Once the RNA payload reaches the target cells, it must egress the endosomal confinement to access the cytoplasm, where it operates on the expression machinery[361]. The endosomal release poses a significant risk of almost total loss of the delivered RNA molecules such as siRNAs. Approximately 99% of free siRNA and ASOs remain confined within endosomal membranes, with the evasively released portion eliciting an RNAi response in the cytoplasm[36,101,404,405].

#### 4.2.4. *Non-specific immunogenicity*

Applying mRNA vaccines in cancer immunotherapy emphasizes the immune−stimulatory activity of mRNA that can act as a self-adjuvant by inducing type I IFNs[406]. This immunogenicity is mainly mediated through the activation of TLRs. However, immunogenicity has a downside since it reduces the expression of the target protein by increasing mRNA clearance, which is against the desired clinical outcome[34]. Earlier, short siRNA duplexes were assumed to be immunoevasive. Later, it was proven that introducing foreign RNA into the body can mediate immune responses, leading to the delivered RNA clearance or type III IFN-mediated inflammation[363]. Administering siRNAs in non-conjugated form or without nanoparticle complexes brings in rapid kidney filtration within a few minutes with negligible circulation[407]. In this case, camouflaging techniques should be adopted to tackle the immunogenicity against RNA molecules[408,409].

In recent years, there has been evidence of a link between the structural properties of siRNAs (length, backbone, and sequence) and irAEs. A higher immunologic response can be observed for GU-rich sequences, ribose skeleton, and sequences $\geq$19-nucleotide in length. Moreover, the therapeutic potential of siRNA is marred by the non-specific immune stimulation, rendering it challenging to discern the therapeutic outcomes. Recognizing and tackling these unintended immune responses is necessary for optimizing the safety and efficacy of siRNAs in cancer treatment.

#### 4.2.5. *Off-target effects*

In addition to the common challenges for RNA therapies, precise and carefully designed sequences are crucial for siRNAs introduced to silence targeted genes, to avoid toxicity and IFN-mediated responses[363]. A defective siRNA design causes them to behave similarly to miRNAs, thereby interfering with or degrading mRNAs owing to the complementarity between the 3′UTR of unintended mRNAs and the 5′ end guide strand of siRNA[410]. These miRNA-like off-target effects potentially impair the on-target responses and, thereby, therapeutic efficiency, potentially giving rise to observed phenotypic changes. Non-specific adherence becomes a question when siRNA recognizes eleven nucleotide sequences in common between target and non-target genes[411]. Moreover, therapeutic siRNAs would likely compete with endogenous miRNAs for RISC, which may exacerbate the potential off-target effects[412]. Since these effects emerge in a dose-dependent manner, reducing siRNA doses. Also, optimizing siRNA design or siRNA pooling at lower concentrations might evade the adverse events of complementarity[413].

Similarly, virally engineered CAR-T-cell therapies pose off-target effects due to cross-reactivity to unintended antigens. As a result, serious neurotoxicity, cardiotoxicity, and anaphylactic reactions may occur, as well as complications related to immunosuppression[364]. While transfecting T-cells with mRNA may alleviate these effects, the potential risks should be closely monitored.

### 4.3. *Vector-related challenges*

#### 4.3.1. *Size, surface charge and tunability*

An optimized nanocarrier design must consider characteristics such as size, shape, surface charges, decoration, and their influence on tumor uptake and accumulation. It is tricky to tune the optimal particle size for sustained circulation and effective uptake since very small particles may clear quickly, while larger particles may not deliver the payload effectively[414]. Furthermore, particle morphology distribution in target organs is another fundamental factor associated with cellular uptake and biodistribution, particularly in the TME. Particles can be present in several forms, such as nanospheres, nanodiscoids, nanotubes, and nanorods[415,416]. When it comes to elongated particles such as carbon nanotubes and gold nanorods, it is important to consider the aspect ratio, which should be reduced to enhance the efficiency of those particles[191].

Multiple factors govern the nanoparticle transfer across the microvascular networks to malignant tissue. In addition to the EPR effect, particle size, morphology, and surface properties play

a role in vector accumulation and delivery efficiency, plus the dynamic nature of the tumor vasculature, as discussed earlier. The leaky nature of tumor vasculature offers passive uptake and retention opportunities for spherical nanoparticles with diameters near 100 nm[417]. As for elongated particles, such as discoidal nanoparticles or nanorods, they may accumulate through adhesion to tumor vessels or binding to active ligands. Nevertheless, simulation data show the superiority of spherical and cuboidal nanoparticles over rod and discoidal forms[418]. Meanwhile, filamentous particles tend to extravasate and accumulate within tumors despite their large size, thanks to their tiny radius[417].

Surface charges could exert an effect on particles' uptake, biodistribution, and interaction with immune cells. Negative-charged nanoparticles are difficult to absorb by cells and achieve adequate biodistribution as they are electrostatically repelled by cell membranes, whereas their cationic counterparts can induce cytotoxicity by compromising cell membrane integrity[419]. Furthermore, a delicate balance must be achieved while modifying nanocarrier surfaces to enhance biocompatibility, mitigating interaction with immune cells, and maximizing cellular uptake while preserving the carriers' desired. Also, careful consideration of potential aggregation, change in nanoparticle fate, and pharmacokinetics.

#### 4.3.2. *Safety*

Safety issues involve the assessment of potential unintended off-target effects or severe immune reactions to guarantee that both the payload and the vector do not pose a risk to patients. Non-viral vectors are safer options with larger capacity. Yet, concerns about toxicity arise due to the size, cationic charges, and complexity of preparing these nanocomplexes and the need for recurring exposure[420]. Treating chronic conditions will require prolonged administration of mRNA lipoplexes or polyplexes, where serious concerns remain regarding toxicities associated with cationic lipids or polymers[211]. Moreover, efficient delivery of negatively charged nucleic acid to cells requires cationic nanomaterial that can embrace this macromolecule and transfer it to the target. Positively charged nanomaterials may be toxic to the body since they exert nonspecific interactions with non-relevant cellular targets[421]. Therefore, tuning RNA release in a controlled manner and sustaining the gene expression or knockdown levels is critical when a temporary effect is desired.

#### 4.3.3. *Biocompatibility and biodegradability*

Creating effective nanocarriers for RNA entrapment and release hinges on finding the harmony between achieving effective transfection and biocompatibility with minimum unwanted cellular responses. Nanoparticle cytotoxicity is pertinent to their shape and charges. Cationic nanoparticles, including LNPs and PEIs-coated nanoparticles, impact cellular pathways as they exert proapoptotic and proinflammatory activities while arousing membrane protein destabilization[173]. Even biocompatible polymers like PLGA or reducible polymers may not guarantee that RNA molecules will be released and interact successfully while achieving effective transfection efficiency[422]. A further challenge is the cell-damaging effects attributable to nanoparticle shape, which could extend to the genotoxic effects as reported with SWCNTs by Kisin et al[423,424].

A downside of nanomaterials like traditional MSNs is their slow degradation rate, which raises concerns about accumulation and toxicity in the long term[180]. If left unchecked, this persistence can accumulate silica nanoparticles in tissues and organs, potentially resulting in health complications. Hence, to reduce potential risks associated with prolonged retention of common MSNs in the body, alternative silica-based nanostructures, such as DMSNs, have been developed for gene delivery. On the other hand, DMSNs offer enhanced biodegradability offered by dendritic structures, which reduces their bioaccumulation in the body[425]. In addition, it is pertinent to acknowledge the non-biodegradability of fullerenes and CNTs, even with their promise in gene delivery[426]. Although several attempts have been made to hydrophilize fullerenes, they remain prone to accumulation in the cytoplasm due to their hydrophobic structure. Consequently, fullerenes may build up in vital organs, whereas CNTs' intrinsic stability raises doubts concerning long-term safety. Use of these carbon structures necessitates modifications to develop biodegradable and biocompatible options with expedited clearance from tissues and organs where effective degradation pathways are absent.

### 4.4. *Routes of administration*

The administration route through which RNA formulations, whether systemically or locally, plays a critical role in determining their performance and efficacy. The administration of RNA therapeutics is facing several challenges, as summarized as follows: Intranodal or intratumoral injections are often used and hold promise for RNA cancer immunotherapeutics, but precision is crucial to avoid improper tissue infusion. Intratumoral administration faces challenges with limited reach to distant tumors, tumor variability, and immunosuppressive microenvironments. Intravenous delivery, while efficient, encounters rapid clearance, poor biodistribution, and the need for recurrent exposures. Intramuscular injections, advantageous for vaccines, may induce inflammation, risking patient compliance.

Intradermally and subcutaneously delivered RNA therapeutics leverage Langerhans cells present locally for effective antigen presentation. It is, however, complex to reach even immune responses across diverse racial and ethnic groups due to differences in skin thickness, vascularity, and tissue characteristics[427]. Also, it remains difficult to ensure consistency and targeting efficiency of RNA delivery by locally implantable hydrogels[428]. For successful intradermal administration, the dose should be precisely injected between the dermis and epidermis by skilled personnel for accuracy. However, the patient may experience discomfort due to local swelling and redness, affecting medication adherence.

The nasal delivery has been praised for the ease of self-administration, non-invasive nature, high vascularity, and escaping first-pass metabolism, along with the ability to circumvent biological barriers by directly accessing the brain through the olfactory pathway[429]. Nevertheless, optimizing brain delivery while keeping the lungs unexposed is not a simple task. Aside from that, this route is limited by limited dose volumes, RNA degradation by nasal enzymes, and removal *via* ciliary movement. This contributes to inadequate absorption due to brief dose retention, and in case repeated instillations would still produce uneven doses.

On the other hand, inhalable mRNA formulations present numerous technical difficulties, including physical and biological barriers, stability, accurate dosage, and clinical assessment. The lung mucosa is covered with negatively charged mucin, potentially exacerbating particle instability, aggregate formation, and confinement in this layer. In this regard, introducing formulations with negative outer-layer or PEG coatings confers their ability to repel mucin components, showing enhanced mucosal stability[430].

It is also hard to develop a predictive model to characterize inhalation therapies through *in vitro* and *in vivo* correlations or computational modelling. A connection could be made to study the crucial airway-on-a-chip models, emphasize aerosol deposition in 3D cultures, and underline the need to enhance physiologically based pharmacokinetic models for formulation development[431]. To evade the toxicity incurred by the inhalation route, direct instillation to bronchial proves an alternative rapid and efficient delivery approach[432].

Oral administration is the most patient-friendly, with great adherence and tolerability, yet this route is burdened with formidable limitations. When administered orally, nanoparticles, especially LNPs, are exposed to an unprecedented environment, including the acidic pH, digestive enzymes, and viscous mucin barrier layer. Gastric acidity, which reaches around pH 1.5, can negatively affect endosome-responsive systems such as ionizable lipids, CARTs, and other polymer substances. Additionally, pancreatic lipases quickly degrade lipids-based delivery systems, and a set of polymeric materials are also esterase-sensitive, leading to the premature release and loss of the RNA payload[433].

The local administration route is renowned for sustained delivery capabilities, which can resolve the rapid clearance rate and bypass cellular barriers by creating alternative pathways and harnessing nanoparticle permeability to their intended destination[434]. Local administration requires non-immunogenic hydrogels for perpetual RNA release. Microneedles offer enhanced patient compliance for transdermal delivery but face safety concerns and limited clinical viability.

Hydrodynamic gene delivery uses high-pressure injection of DNA solution to permeabilize capillary endothelium, allowing nucleic acids to enter parenchymal cells[435]. Building on this, Dul et al.[436] explored MicronJet600®, a microneedle device, for intradermal pDNA delivery in the human breast skin. MicronJet creates superficial microdisruptions, enabling gene expression in the epidermis and dermis with minimal damage, promising better results than conventional injections. The hydrodynamic delivery approach, while promising, raises safety concerns and lacks sufficient evidence in clinical cancer therapy.

Despite the promising results in preclinical studies, overcoming challenges in RNA therapeutic administration remains crucial for widespread clinical viability. Safety concerns around the large injection volumes that might lead to probable transient heart failure, with a risk of tissue damage, imprecise control of the hydrodynamic pressure, and interpatient variability. Addressing safety concerns, improving precision, and advancing delivery technologies are essential for realizing the full potential of RNA therapeutics in cancer treatment.

### 4.5. *Combination therapy*

mRNA cancer vaccines and other RNA therapies can be conjugated with conventional tumor therapies, including chemotherapy, radiotherapy, and immunotherapy. Monotherapy with chemotherapies, such as cytokine monotherapy, targets only a few of the mechanisms controlling tumor growth and metastasis, resulting in modest antitumor responses and high toxicity[437]. Cancer progression has proven to be challenging for some metastatic solid tumors, such as breast cancer, which calls for complementary interventions to address these complexities[438]. The integration of various adjuvants and therapeutic modalities forms the cornerstone of combination therapy in cancer immunotherapy, especially involving mRNA-based approaches. The strategy of co-delivery leverages the synergistic effects of different pathways and mechanisms to enhance T-cell activation and overcome immune tolerance[439]. As well, combination mRNA vaccines, as multiple antigens generated from single or multiple strands, offer opportunities for enhancing therapeutic outcomes.

A rational combination approach would be to use adjuvant mRNA cancer vaccines with ICIs to jointly boost the immune response to fight malignancies. Inhibitory signals blockade by ICIs (*e.g.*, CTLA-4 and PD-1/PD-L1 antagonists) promotes T-cell activation in response to mRNA vaccines[440]. TriMix, an adjuvant immunotherapy composed of three naked mRNAs, activates $CD8^+$ and $CD4^+$ T-cells by triggering the production of CD40 and CD40L, along with improving DC antigen presentation by generating constitutively active TLR4[441]. Clinical trials have demonstrated the effectiveness of TriMix mRNA formulations in treating stage III/IV melanoma, either alone or in combination with CPIs. By overcoming immune ICIs, this combination enhances T-cell responses, thereby boosting immune activation pathways and sensitizing tumors to T-cell-mediated killing. This would ultimately aid in tumor control and prolong survival.

In the clinical context of local radiotherapy, the protamine-based mRNA (CV9202) has demonstrated robust translation efficiency, resulting in elevated expression of antigens, triggering immunity against a spectrum of non-small cell lung cancers[442]. Radiation can induce immunogenic cell death, which promotes immune recognition and infiltration into the TME. Inversely, RNAi could potentiate the immune response of traditional cancer therapeutics hampered by certain ICIs, as was the case of CDK inhibitors and PDL-1 siRNA[118]. Furthermore, siRNA has been shown to increase the efficacy of photodynamic therapy in cancer immunotherapy. For instance, knocking down PD-L1 counteracts the immunosuppressive effects of the TME on T-cell antitumor immune responses[114,443].

Moreover. a synergistic therapeutic effect was observed when mRNA vaccines were co-administered with routine chemotherapy such as cisplatin and durvalumab. Infiltration of immunosuppressive cells such as Tregs and MDSCs into genital tract malignancies interferes with the ability of the E7-TriMix mRNA vaccine to stimulate immunity against the tumor. Bialkowski coupled mRNA vaccination with cisplatin, which turned out to be highly effective in fully eradicating neoplasms in 88% of cases[444]. Moreover, mRNA-2752, encoding OX40L, was administered in conjugation with durvalumab for improving T-cell response along with IL-23 and IL-36$\gamma$ interleukins and has shown safety and tolerability, inducing sustained pro-inflammatory effects[445].

Even though these combinations face several challenges that are worthy of careful consideration. To gain regulatory approval, these lot-consistent vaccines in a synergistic cocktail need to be clinically evaluated to ensure immunogenicity and tolerability through unbiased clinical trials with defined endpoints[365]. Besides, a potential increased immune response might arise from increased overall mRNA dose, or their co-delivery raises doubts regarding safety and tolerability. It is also difficult to coordinate the optimum administration sequence, timing, and dosing of mRNA cancer vaccines with radiotherapy since, at specific doses, radiotherapy may exert a potential immune suppressant effect[446]. Moreover, combination therapy is also plagued by inter-individual variability, as seen in the combination of GTI-2040, oxaliplatin, and capecitabine, which are used to treat metastatic solid malignancies[447]. Therefore, promising combination therapy has to be universally applicable to different populations. As chemotherapy can have immunosuppressive effects, it is critical to understand

how this might contradict mRNA vaccines' immune activation, as it might terminate with impairing the immune response. This was particularly evident when BNT162b2 was co-administered with chemotherapeutics for cancer patients. Also, the immunosuppressive action did not respond to adjustments in the timing of vaccine administration with on-cycle and off-cycle chemotherapeutics[448]. Hence, dose optimization is necessary to balance the subsequent immunostimulatory and immunosuppressive effects to reach high therapeutic efficacy while minimizing the undesired events.

### 4.6. *Regulatory hurdles*

Most RNA formulations are still in their infancy phase, and the uncertainty surrounding their regulatory approval for clinical applications is a stumbling block to their development. The application of gene therapy, initially pursued for genetic diseases, swiftly transitioned into a prominent approach for cancer treatment, with about two-thirds of gene therapy trials focusing on diverse cancer types[449,450]. The advancement of cancer gene therapy (CGT) products with a scaling-up process requires addressing regulatory hurdles to ensure the effectiveness, safety, and tolerability of this innovative therapeutic approach.

During the development of CGT products, compliance with Chemistry, Manufacturing, and Controls (CMC) regulations is necessary to maintain safety, quality, and efficacy[451]. Throughout the entire development process of CGT products, a comprehensive understanding and disclosure of all CGT product components, including vectors, cells, plasmids, and associated reagents, is necessary, along with rigorous quality control procedures. Stringent regulations for CGT call for a comprehensive description of vectors, cells, and reagents. Therefore, to ensure a safe and reliable final product, the quality control of research-grade reagents, and meeting the demand for RNase-free processing, adherence to current good manufacturing practice (cGMP) standards is imperative, necessitating the precise documentation and execution of the manufacturing process[452]To accomplish this, well-defined criteria for CGT product specifications and materials should be established, with certificates of analysis serving as checkpoints for acceptance. Identity, purity, and safety testing should be conducted, and accurate labeling guarantees a high-quality and impurity-free product. Further microbiological testing ensures sterility and excludes potential biological contamination.

Nevertheless, the regulatory approval process is involved and requires consent from multiple regulatory bodies, such as FDA and the National Institutes of Health's Office of Biotechnology Activities (NIH/OBA)[453]. To prevent delays associated with clinical investigation, it is crucial to communicate regularly with the FDA through pre-IND meetings and other formal engagements to align with regulatory expectations and address unforeseen issues[452]. However, the lack of harmony between regulatory bodies further hampers establishing universally accepted standards. Also, mRNA vaccines and other RNAi therapeutics rely on new technologies, and the production processes are distinct from those of conventional ones; therefore, setting specific quality standards and guidelines is complex.

#### 4.6.1. *Challenges in pre-clinical development*

Contrary to conventional drugs, there is no universal protocol for preclinical evaluation of CGT products. The variety of CGT products and vectors necessitates specific regulations for each class. Also, the nature of the cell source for cellular vaccines necessitates a record of mobilization protocols, collection techniques, and eligibility of donors. To deal with this challenge, the FDA embraced flexibility in line with the CGT product's biology and intended use, along with the evaluation of safety and toxicity for raw materials and the final formulation[366]. Choosing an appropriate animal species and model for a pre-clinical study is problematic due to interspecies variability, which may make it difficult to generalize results to clinical use in humans. Additionally, RNA-nanoparticle biodistribution, ability to bypass biological barriers, and affinity to specific tissue vary according to size, outer surface characteristics, and interaction with plasma proteins[454]. This might inform their tissue-specific accumulation into affinity, which eventually affects the *in vivo* fate and side effects. The multidisciplinary nature of pre-clinical CGT studies emphasizes the need for coordinated efforts of formulation experts, biologists, toxicologists, and regulatory experts to address complicated tasks such as assessing off-target effects and irAEs.

#### 4.6.2. *Challenges in clinical development*

Moving forward with the clinical evaluation of CGT, preclinical and clinical data should be bridged together to avoid unexpected toxicities. In the course of clinical development, CGT products will inevitably encounter scalability limitations. Moreover, any further changes to the CGT production process may require further preclinical studies. It is essential to know if critical quality attributes (CQAs) were altered when modifying processes or facilities during production. Performing *in vitro* and *in vivo* might be necessary to assure efficacy and safety.

In early-phase trials of CGT, efficacy and tolerability are more prominently considered. Careful monitoring of adverse events is required to mitigate potential toxicities emerging from CGT, vector, sustained biological effects, or irAEs. Aside from that, achieving effective dosing and using a highly adherable administration route that does not cause patient noncompliance is important for the developmental clinical phase. A dose-escalating approach might be adopted to determine the effective dose since there is a lack of knowledge of the conversion factor for CGT products. It is also imperative to conduct well-planned phase 2 trials to estimate CGT's activity and demonstrate therapeutic outcomes without rushing through this stage so as to obtain sufficient findings as proof for moving forward to the subsequent trial phase. It is also difficult to select the study population and decide on primary and secondary immunological endpoints for phase III trials. Alignment of these endpoints with improvements in patient survival or function is crucial at the final developmental stage[366].

## 5. Future directions and conclusions

Innovations in RNA therapeutics are gaining traction and will be a great boon for future cancer treatment. Yet, the journey from preclinical to clinical translation in cancer treatment is laden with hurdles that extend far beyond the laboratory bench. Regarding RNA-based therapeutics, scientists are currently actively addressing the lacunae in understanding how to optimize RNA stability and enhance cellular uptake and endosomal egress while minimizing immunogenicity, aiming to reshape the landscape of therapeutic efficacy in cancer therapy. Thus, speeding up the transition of mRNA-based products to market requires extensive optimization effort, which goes beyond just the acquisition of the right technology. Also, achieving adequate tumor accumulation remains an obstacle to successful cancer treatment. Therefore, a key aspect of future endeavors lies in maximizing the potential of

nanoplatforms to interact selectively with malignant tissues with precision, thereby refining cellular uptake mechanisms.

When developing mRNAs, careful design of promoter sequences, UTRs, and poly(A) tails, as well as optimizing coding sequences to enhance stability and translation efficiency, lead to considerable early-stage expenses. Therefore, addressing inherent cost constraints throughout the production phases of mRNA therapeutics is key to success. Optimizing mRNA sequences by LinearDesign, an algorithm to unlock highly stable coding and non-coding regions, provides a faster and more cost-effective alternative to traditional codon optimization, pointing to a promising future[402].

Scalability and cost-effectiveness are key issues that should be addressed to fully realize the potential of RNA therapeutics. Modification of the encoded genes has been introduced to reduce the costs of mRNA. Conventional and self-amplifying mRNA (saRNA) structures have been studied for vaccine development. Although conventional mRNA has a simple structure and is relatively safe, it may necessitate higher doses to achieve optimal results because of its short-lived nature and temporary effects. Meanwhile, by introducing the gene-encoding viral replicase, saRNA can produce high antigen expression levels at significantly lower doses with improved safety (Fig. 15). However, saRNAs still potentially pose safety issues that emerged with the generation of virus-like particles owing to the viral replicase[455]. Recent advances in saRNA technology have led to the creation of *trans*-amplifying mRNA (taRNA), which applies two RNAs: one encoding the replicating gene and the other encoding a non-replicating replicase (Fig. 15). By designing taRNAs with short RNA sequences, yield and quality can be improved.

Addressing both pre- and post-transcriptional challenges posed by the TME to intracellular nucleic acid delivery calls for strategic delivery approaches *via* well-designed nanoplatforms. While PEI has been praised as a rising star polymer for gene delivery, cytotoxicity remains a concern. Recently, cyclic polymers are expected to play an increasingly important role in gene delivery in the future since their distinctive topology allows them to be tunable and less toxic[158]. For example, the sun-shaped PDMAEMA and cyclic PEI demonstrate a shift towards high-performance DNA vectors that open up new avenues of gene delivery[456]. Beyond that, the need for long-term formulation stability, especially for mRNA, is still unresolved, which may necessitate the reliance on DMSNs, and protamine-based delivery systems can provide a promising shelf-life for RNA therapeutics[457,458].

Moreover, there is a necessity for a forward-looking strategy that could enable improved treatment outcomes for extended periods and address the concerns encountered with extrahepatic organ-targeted lipid-based delivery. Introducing SORT molecules to develop LNPs with unique targetability represents a leap in delivery precision and gene transfection at the intended site. Nanomotors had been fabricated to deliver siRNA[459]; therefore, the future may witness the advancement with MSN-based nanobots for RNA delivery that can target hard-to-reach organs like bladder cancer[460]. Finally, the RNA delivery to the target is not the sole challenge; the route of administration also requires advancements. The search for a less invasive and more compliant administration route may be met by the dependence on microneedle patches or inhalable formulations on account of the oral route due to the associated formidable obstacles.

In conclusion, it is clear that RNA-based therapy has the potential to be very effective at treating a wide range of malignancies. In the coming years, the adaptability of mRNA technology and the lessons learned from the widespread use of mRNA vaccines during the recent pandemic are expected to propel further advances in personalized cancer vaccines. As well, RNAi-mediated immunostimulation has been found effective in fighting cancer immune evasion, and several genetic tools can be used to achieve this goal. Thanks to advances in nanobiotechnology, it has become possible to meet the essential needs for gene delivery and targetability. In addition to looking at how

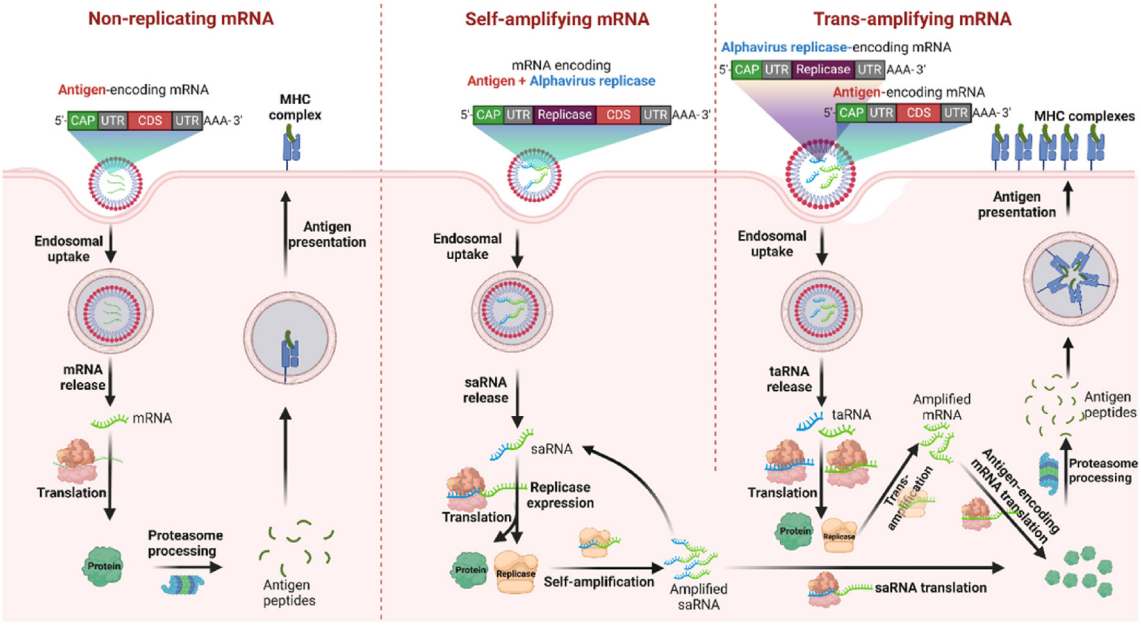


**Figure 15** Protein expression and antigen presentation using mRNA, saRNA, and taRNA approaches. AAA: Poly A tail, CAP: Capping sequence, CDS: Coding sequence, MHC: Major histocompatibility complex, UTR: untranslated region. Figure was made by Biorender.com.

nanoplatforms are effective and safe, it is also important to consider how they break down and to consider how they are designed to be biodegradable and multifunctional. RNA immunotherapeutics have yet to realize their potential as they are confronted with unprecedented obstacles relating to either the molecules themselves or the nature of the malignant tumor. Furthermore, there are several regulatory issues to deal with until the product is marketed. Taking steps to overcome these obstacles could result in the approval of future RNA-based cancer treatments.

## Acknowledgments

Hongjie An acknowledges the support from the Australian Research Council Future Fellowship (No. FT180100361, Australia) and Discovery Project (No. DP230100556, Australia). Nam-Trung Nguyen thanks the support from Australian Laureate Fellowship (FL230100023, Australia).

## Author contributions

Mohamed S. Attia: Writing − review & editing, Writing − original draft, Conceptualization. Gregor Kijanka: Writing − review & editing. Nam-Trung Nguyen: Writing − review & editing. Jun Zhang: Writing − review & editing, Supervision. Hongjie An: Writing − review & editing, Supervision, Funding acquisition.

## Conflicts of interest

The authors declare no conflict of interest.

## References

1. Schuster M, Nechansky A, Kircheis R. Cancer immunotherapy. *Biotechnol J* 2006;**1**:138−47.
2. Tamura F, Sato Y, Hirakawa M, Yoshida M, Ono M, Osuga T, et al. RNAi-mediated gene silencing of ST6GalNAc I suppresses the metastatic potential in gastric cancer cells. *Gastric Cancer* 2016;**19**:85−97.
3. Mockey M, Bourseau E, Chandrashekhar V, Chaudhuri A, Lafosse S, Le Cam E, et al. mRNA-based cancer vaccine: prevention of B16 melanoma progression and metastasis by systemic injection of MART1 mRNA histidylated lipopolyplexes. *Cancer Gene Ther* 2007;**14**:802−14.
4. Oiseth SJ, Aziz MS. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. *J Cancer Metastasis Treat* 2017;**3**:250−61.
5. Busch W. Aus der Sitzung der medicinischen Section vom 13 November 1867. *Berl Klin Wochenschr* 1868;**5**:137.
6. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. 1. *Am J Med Sci* 1893;**105**:487.
7. Coley WB. The treatment of sarcoma with the mixed toxins of erysipelas and bacillus prodigiosus. *Boston Med Surg J* 1908;**158**:175−82.
8. Morales A, Eidinger D, Bruce A. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. *J Urol* 1976;**116**:180−2.
9. Ehrlich P. *Ueber den jetzigen Stand der Karzinomforschung. Beiträge zur experimentellen Pathologie und Chemotherapie*. Leipzig: Akademisch Verlagsgesellschaft m.b.H.; 1909. p. 117−64.
10. Burnet M. Cancer—a biological approach: III. Viruses associated with neoplastic conditions. IV. Practical applications. *BMJ* 1957;**1**:841.
11. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol* 2020;**17**:807−21.
12. Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. *Mol Cancer* 2021;**20**:1−23.
13. Fahey JL. Cancer in the immunosuppressed patient. *Ann Intern Med* 1971;**75**:310−2.
14. Maize JC. Skin cancer in immunosuppressed patients. *JAMA* 1977;**237**:1857−8.
15. Chehelgerdi M, Chehelgerdi M. The use of RNA-based treatments in the field of cancer immunotherapy. *Mol Cancer* 2023;**22**:106.
16. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov* 2018;**17**:261−79.
17. Ostro MJ, Giacomoni D, Lavelle D, Paxton W, Dray S. Evidence for translation of rabbit globin mRNA after liposomemediated insertion into a human cell line. *Nature* 1978;**274**:921−3.
18. Melton D, Krieg P, Rebagliati M, Maniatis T, Zinn K, Green M. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res* 1984;**12**:7035−56.
19. Martinon F, Krishnan S, Lenzen G, Magné R, Gomard E, Guillet JG, et al. Induction of virus-specific cytotoxic T lymphocytes *in vivo* by liposome-entrapped mRNA. *Eur J Immunol* 1993;**23**:1719−22.
20. Dolgin E. The tangled history of mRNA vaccines. *Nature* 2021;**597**:318−24.
21. Qin S, Tang X, Chen Y, Chen K, Fan N, Xiao W, et al. mRNA-based therapeutics: powerful and versatile tools to combat diseases. *Signal Transduct Target Ther* 2022;**7**:166.
22. Heiser A, Coleman D, Dannull J, Yancey D, Maurice MA, Lallas CD, et al. Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. *J Clin Invest* 2002;**109**:409−17.
23. Bajan S, Hutvagner G. RNA-Based Therapeutics: from antisense oligonucleotides to miRNAs. *Cells* 2020;**9**:137.
24. Costa B, Boueri B, Oliveira C, Silveira I, Ribeiro AJ. Lipoplexes and polyplexes as nucleic acids delivery nanosystems: the current state and future considerations. *Expert Opin Drug Deliv* 2022;**19**:577−94.
25. Zhang Y, Lu L, Song F, Zou X, Liu Y, Zheng X, et al. Research progress on non-protein-targeted drugs for cancer therapy. *J Exp Clin Cancer Res* 2023;**42**:62.
26. Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the messenger: advances in technologies for therapeutic mRNA delivery. *Mol Ther* 2019;**27**:710−28.
27. Takeshita F, Ochiya T. Therapeutic potential of RNA interference against cancer. *Cancer Sci* 2006;**97**:689−96.
28. Yuan Y, Gao F, Chang Y, Zhao Q, He X. Advances of mRNA vaccine in tumor: a maze of opportunities and challenges. *Biomark Res* 2023;**11**:6.
29. Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* 2017;**543**:248−51.
30. Thomas SJ, Moreira Jr ED, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. *N Engl J Med* 2021;**385**:1761−73.
31. Mu X, Hur S. Immunogenicity of *in vitro*-transcribed RNA. *Acc Chem Res* 2021;**54**:4012−23.
32. Weng Y, Li C, Yang T, Hu B, Zhang M, Guo S, et al. The challenge and prospect of mRNA therapeutics landscape. *Biotechnol Adv* 2020;**40**:107534.
33. Eberhardt W, Doller A, Akool ES, Pfeilschifter J. Modulation of mRNA stability as a novel therapeutic approach. *Pharmacol Ther* 2007;**114**:56−73.

34. Lee J, Woodruff MC, Kim EH, Nam JH. Knife's edge: balancing immunogenicity and reactogenicity in mRNA vaccines. *Exp Mol Med* 2023;**55**:1305−13.
35. Mulroney TE, Pöyry T, Yam-Puc JC, Rust M, Harvey RF, Kalmar L, et al. N 1-methylpseudouridylation of mRNA causes+ 1 ribosomal frameshifting. *Nature* 2024;**625**:189−94.
36. Dowdy SF, Setten RL, Cui XS, Jadhav SG. Delivery of RNA therapeutics: the great endosomal escape. *Nucleic Acid Ther* 2022;**32**: 361−8.
37. von Niessen AGO, Poleganov MA, Rechner C, Plaschke A, Kranz LM, Fesser S, et al. Improving mRNA-based therapeutic gene delivery by expression-augmenting 3′ UTRs identified by cellular library screening. *Mol Ther* 2019;**27**:824−36.
38. Kim SC, Sekhon SS, Shin W-R, Ahn G, Cho B-K, Ahn J-Y, et al. Modifications of mRNA vaccine structural elements for improving mRNA stability and translation efficiency. *Mol Cell Toxicol* 2022;**18**: 1−8.
39. Leppek K, Das R, Barna M. Functional 5′ UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nat Rev Mol Cell Biol* 2018;**19**:158−74.
40. Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M. High guanine and cytosine content increases mRNA levels in mammalian cells. *Plos Biol* 2006;**4**:e180.
41. Kallen KJ, Theß A. A development that may evolve into a revolution in medicine: mRNA as the basis for novel, nucleotide-based vaccines and drugs. *Ther Adv Vaccin* 2014;**2**:10−31.
42. Mauger DM, Cabral BJ, Presnyak V, Su SV, Reid DW, Goodman B, et al. mRNA structure regulates protein expression through changes in functional half-life. *Proc Natl Acad Sci U S A* 2019;**116**:24075−83.
43. Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J Control Release* 2015;**217**:337−44.
44. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* 2005;**23**: 165−75.
45. Markov O, Oshchepkova A, Mironova N. Immunotherapy based on dendritic cell-targeted/-derived extracellular vesicles—a novel strategy for enhancement of the anti-tumor immune response. *Front Pharmacol* 2019;**10**:1152.
46. Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: the next immunotherapy frontier. *Nat Cancer* 2022;**3**:911−26.
47. Roney MMSI, Lanagan MC, Sheng Y, Gough MM, Snell C, Nguyen NT, et al. Isotypic analysis of anti-p53 serum autoantibodies and p53 protein tissue phenotypes in colorectal cancer. *Hum Pathol* 2022;**128**:1−10.
48. Zhao Y, Baldin AV, Isayev O, Werner J, Zamyatnin AA, Bazhin AV. Cancer vaccines: antigen selection strategy. *Vaccines* 2021;**9**:85.
49. Wei J, Hui AM. The paradigm shift in treatment from Covid-19 to oncology with mRNA vaccines. *Cancer Treat Rev* 2022;**107**:102405.
50. Lorentzen CL, Haanen JB, Met Ö, Svane IM. Clinical advances and ongoing trials on mRNA vaccines for cancer treatment. *Lancet Oncol* 2022;**23**:E450−8.
51. Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: promising targets for cancer therapy. *Signal Transduct Target Ther* 2023;**8**:1−38.
52. Fiedler K, Lazzaro S, Lutz J, Rauch S, Heidenreich R. mRNA cancer vaccines. *Recent Results Cancer Res* 2016;**209**:61−85.
53. Medrano RFV, Hunger A, Mendonça SA, Barbuto JAM, Strauss BE. Immunomodulatory and antitumor effects of type I interferons and their application in cancer therapy. *Oncotarget* 2017;**8**:71249−84.
54. Mortezaee K, Majidpoor J. Roles for macrophage-polarizing interleukins in cancer immunity and immunotherapy. *Cell Oncol* 2022; **45**:333−53.
55. Ozga AJ, Chow MT, Luster AD. Chemokines and the immune response to cancer. *Immunity* 2021;**54**:859−74.
56. De Lombaerde E, De Wever O, De Geest BG. Delivery routes matter: safety and efficacy of intratumoral immunotherapy. *Biochim Biophys Acta Rev Cancer* 2021;**1875**:188526.
57. Radomski M, Zeh HJ, Edington HD, Pingpank JF, Butterfield LH, Whiteside TL, et al. Prolonged intralymphatic delivery of dendritic cells through implantable lymphatic ports in patients with advanced cancer. *J Immunother Cancer* 2016;**4**:1−9.
58. Ding Y, Li Z, Jaklenec A, Hu Q. Vaccine delivery systems toward lymph nodes. *Adv Drug Deliv Rev* 2021;**179**:113914.
59. Weber JS, Carlino MS, Khattak A, Meniawy T, Ansstas G, Taylor MH, et al. Individualised neoantigen therapy mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab monotherapy in resected melanoma (KEYNOTE-942): a randomised, phase 2b study. *Lancet* 2024;**403**:632−44.
60. Kallen K-J, Heidenreich R, Schnee M, Petsch B, Schlake T, Thess A, et al. A novel, disruptive vaccination technology: self-adjuvanted RNActive® vaccines. *Hum Vaccin Immunother* 2013;**9**:2263−76.
61. Uchida S, Yoshinaga N, Yanagihara K, Yuba E, Kataoka K, Itaka K. Designing immunostimulatory double stranded messenger RNA with maintained translational activity through hybridization with poly A sequences for effective vaccination. *Biomaterials* 2018;**150**:162−70.
62. Butkowsky C, Aldor N, Poynter SJ. Toll-like receptor 3 ligands for breast cancer therapies. *Mol Clin Oncol* 2023;**19**:1−8.
63. Sebastian M, Papachristofilou A, Weiss C, Früh M, Cathomas R, Hilbe W, et al. Phase Ib study evaluating a self-adjuvanted mRNA cancer vaccine (RNActive®) combined with local radiation as consolidation and maintenance treatment for patients with stage IV non-small cell lung cancer. *BMC Cancer* 2014;**14**:1−10.
64. Anderson BR, Muramatsu H, Nallagatla SR, Bevilacqua PC, Sansing LH, Weissman D, et al. Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. *Nucleic Acids Res* 2010;**38**:5884−92.
65. Ting CC, Lavrin DH, Shiu G, Herberman RB. Expression of fetal antigens in tumor cells. *Proc Natl Acad Sci U S A* 1972;**69**:1664−8.
66. Kijanka G, Hector S, Kay EW, Murray F, Cummins R, Murphy D, et al. Human IgG antibody profiles differentiate between symptomatic patients with and without colorectal cancer. *Gut* 2010;**59**:69−78.
67. Kijanka G, Murphy D. Protein arrays as tools for serum autoantibody marker discovery in cancer. *J Proteomics* 2009;**72**:936−44.
68. Peng M, Mo Y, Wang Y, Wu P, Zhang Y, Xiong F, et al. Neoantigen vaccine: an emerging tumor immunotherapy. *Mol Cancer* 2019;**18**: 128.
69. Media Merck. *Merck and Moderna initiate phase 3 study evaluating V940 (mRNA-4157) in combination with KEYTRUDA® (pembrolizumab) for adjuvant treatment of patients with resected high-risk(stage IIB-IV) melanoma*. Available from: https://www.merck.com/news/merck-and-moderna-initiate-phase-3-study-evaluating-v940-mrna-4157-in-combination-with-keytruda-pembrolizumab-for-adjuvant-treatment-of-patients-with-resected-high-riskstage-iib-iv-melanom/, 2023. [Accessed 15 June 2024].
70. Vasudevan SS, Kandrikar TY, Sayyed AA, Sridhar S, Prasad A, Khandelwal S, et al. Personalized vaccines, novel vaccination technologies, and future prospects. In: Chavada VP, Vora LK, Apostolopoulos V, editors. *Advanced vaccination technologies for infectious and chronic diseases*. Amsterdam: Elsevier; 2024. p. 225−42.
71. Yuan J, Hegde PS, Clynes R, Foukas PG, Harari A, Kleen TO, et al. Novel technologies and emerging biomarkers for personalized cancer immunotherapy. *J Immunother Cancer* 2016;**4**:1−25.
72. Katsikis PD, Ishii KJ, Schliehe C. Challenges in developing personalized neoantigen cancer vaccines. *Nat Rev Immunol* 2024;**24**:213−27.
73. Rasmussen M, Fenoy E, Harndahl M, Kristensen AB, Nielsen IK, Nielsen M, et al. Pan-specific prediction of peptide−MHC class I complex stability, a correlate of T cell immunogenicity. *J Immunol* 2016;**197**:1517−24.

74. Jou J, Harrington KJ, Zocca M-B, Ehrnrooth E, Cohen EE. The changing landscape of therapeutic cancer vaccines—novel platforms and neoantigen identification. *Clin Cancer Res* 2021;**27**:689−703.
75. Wells DK, van Buuren MM, Dang KK, Hubbard-Lucey VM, Sheehan KC, Campbell KM, et al. Key parameters of tumor epitope immunogenicity revealed through a consortium approach improve neoantigen prediction. *Cell* 2020;**183**:818−34.
76. Schmidt J, Smith AR, Magnin M, Racle J, Devlin JR, Bobisse S, et al. Prediction of neo-epitope immunogenicity reveals TCR recognition determinants and provides insight into immunoediting. *Cell Rep Med* 2021;**2**:100194.
77. Veatch JR, Jesernig BL, Kargl J, Fitzgibbon M, Lee SM, Baik C, et al. Endogenous $CD4^+$ T cells recognize neoantigens in lung cancer patients, including recurrent oncogenic KRAS and ERBB2 (Her2) driver mutations. *Cancer Immunol Res* 2019;**7**:910−22.
78. Gritstone. Gritstone bio [Internet]. Available from: https://gritstonebio.com/. [Accessed June 15, 2024].
79. Bulik-Sullivan B, Busby J, Palmer CD, Davis MJ, Murphy T, Clark A, et al. Deep learning using tumor HLA peptide mass spectrometry datasets improves neoantigen identification. *Nat Biotechnol* 2019;**37**:55−63.
80. Jaeger AM, Stopfer LE, Ahn R, Sanders EA, Sandel DA, Freed-Pastor WA, et al. Deciphering the immunopeptidome *in vivo* reveals new tumour antigens. *Nature* 2022;**607**:149−55.
81. Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. *Cell Res* 2017;**27**:74−95.
82. Daei Sorkhabi A, Mohamed Khosroshahi L, Sarkesh A, Mardi A, Aghebati-Maleki A, Aghebati-Maleki L, et al. The current landscape of CAR T-cell therapy for solid tumors: mechanisms, research progress, challenges, and counterstrategies. *Front Immunol* 2023;**14**:1113882.
83. Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. *Nat Rev Cancer* 2013;**13**:525−41.
84. Mackensen A, Haanen JB, Koenecke C, Alsdorf W, Wagner-Drouet E, Borchmann P, et al. CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. *Nat Med* 2023;**29**:1−10.
85. Beatty GL, O'Hara MH, Lacey SF, Torigian DA, Nazimuddin F, Chen F, et al. Activity of mesothelin-specific chimeric antigen receptor T cells against pancreatic carcinoma metastases in a phase 1 trial. *Gastroenterology* 2018;**155**:29−32.
86. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol* 2023;**20**:359−71.
87. Lee L, Lim WC, Galas-Filipowicz D, Fung K, Taylor J, Patel D, et al. Limited efficacy of APRIL CAR in patients with multiple myeloma indicate challenges in the use of natural ligands for CAR T-cell therapy. *J Immunother Cancer* 2023;**11**:e006699.
88. Parayath NN, Stephan SB, Koehne AL, Nelson PS, Stephan MT. *In vitro*-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells *in vivo*. *Nat Commun* 2020;**11**:6080.
89. Śledź M, Wojciechowska A, Zagożdżon R, Kaleta B. *In situ* programming of CAR-T cells: a pressing need in modern immunotherapy. *Arch Immunol Ther Exp* 2023;**71**:18.
90. Smith TT, Stephan SB, Moffett HF, McKnight LE, Ji W, Reiman D, et al. *In situ* programming of leukaemia-specific T cells using synthetic DNA nanocarriers. *Nat Nanotech* 2017;**12**:813−20.
91. Gökirmak T, Nikan M, Wiechmann S, Prakash TP, Tanowitz M, Seth PP. Overcoming the challenges of tissue delivery for oligonucleotide therapeutics. *Trends Pharmacol Sci* 2021;**42**:588−604.
92. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998;**391**:806−11.
93. Monia BP, Lesnik E, Gonzalez C, Lima WF, McGee D, Guinosso CJ, et al. Evaluation of 2′-modified oligonucleotides containing 2′-deoxy gaps as antisense inhibitors of gene expression. *J Biol Chem* 1993;**268**:14514−22.
94. Kawasaki AM, Casper MD, Freier SM, Lesnik EA, Zounes MC, Cummins LL, et al. Uniformly modified 2′-deoxy-2′-fluoro-phosphorothioate oligonucleotides as nuclease-resistant antisense compounds with high affinity and specificity for RNA targets. *J Med Chem* 1993;**36**:831−41.
95. Sierant M, Sobczak M, Janicka M, Paduszynska A, Piotrzkowska D. Biological and physicochemical characterization of siRNAs modified with 2′,2′-difluoro-2′-deoxycytidine (gemcitabine). *New J Chem* 2010;**34**:918−24.
96. Fauster K, Hartl M, Santner T, Aigner M, Kreutz C, Bister K, et al. 2′-Azido RNA, a versatile tool for chemical biology: synthesis, X-ray structure, siRNA applications, click labeling. *ACS Chem Biol* 2012;**7**:581−9.
97. Lavergne T, Baraguey C, Dupouy C, Parey N, Wuensche W, Sczakiel G, et al. Synthesis and preliminary evaluation of pro-RNA 2′-O-masked with biolabile pivaloyloxymethyl groups in an RNA interference assay. *J Org Chem* 2011;**76**:5719−31.
98. Hall AHS, Wan J, Shaughnessy EE, Ramsay Shaw B, Alexander KA. RNA interference using boranophosphate siRNAs: structure−activity relationships. *Nucleic Acids Res* 2004;**32**:5991−6000.
99. Efthymiou TC, Huynh V, Oentoro J, Peel B, Desaulniers JP. Efficient synthesis and cell-based silencing activity of siRNAS that contain triazole backbone linkages. *Bioorg Med Chem Lett* 2012;**22**:1722−6.
100. Huang Y, Chen Z, Chen Y, Zhang H, Zhang Y, Zhao Y, et al. Effects of conformational alteration induced by D-/L-isonucleoside incorporation in siRNA on their stability in serum and silencing activity. *Bioconjug Chem* 2013;**24**:951−9.
101. Springer AD, Dowdy SF. GalNAc−siRNA conjugates: leading the way for delivery of RNAi therapeutics. *Nucleic Acid Ther* 2018;**28**:109−18.
102. Braasch DA, Paroo Z, Constantinescu A, Ren G, Öz OK, Mason RP, et al. Biodistribution of phosphodiester and phosphorothioate siRNA. *Bioorg Med Chem Lett* 2004;**14**:1139−43.
103. Rajeev KG, Nair JK, Jayaraman M, Charisse K, Taneja N, O'Shea J, et al. Hepatocyte-specific delivery of siRNAs conjugated to novel non-nucleosidic trivalent *N*-acetylgalactosamine elicits robust gene silencing *in vivo*. *Chembiochem* 2015;**16**:903−8.
104. Sehgal A, Barros S, Ivanciu L, Cooley B, Qin J, Racie T, et al. An RNAi therapeutic targeting antithrombin to rebalance the coagulation system and promote hemostasis in hemophilia. *Nat Med* 2015;**21**:492−7.
105. Ahn I, Kang CS, Han J. Where should siRNAs go: applicable organs for siRNA drugs. *Exp Mol Med* 2023;**55**:1283−92.
106. Ebrahimi N, Manavi MS, Nazari A, Momayezi A, Faghihkhorasani F, Abdulwahid AH, et al. Nano-scale delivery systems for siRNA delivery in cancer therapy: new era of gene therapy empowered by nanotechnology. *Environ Res* 2023;**239**:117263.
107. Xu Z, Wang Y, Zhang L, Huang L. Nanoparticle-delivered transforming growth factor-$\beta$ siRNA enhances vaccination against advanced melanoma by modifying tumor microenvironment. *ACS Nano* 2014;**8**:3636−45.
108. Qiao C, Yang J, Shen Q, Liu R, Li Y, Shi Y, et al. Traceable nanoparticles with dual targeting and ROS response for RNAi-based immunochemotherapy of intracranial glioblastoma treatment. *Adv Mater* 2018;**30**:1705054.
109. Yi AK, Chace JH, Cowdery JS, Krieg AM. IFN-gamma promotes IL-6 and IgM secretion in response to CpG motifs in bacterial DNA and oligodeoxynucleotides. *J Immunol* 1996;**156**:558−64. Baltimore, Md: 1950.
110. Salem AK, Weiner GJ. CpG oligonucleotides as immunotherapeutic adjuvants: innovative applications and delivery strategies. *Adv Drug Deliv Rev* 2009;**61**:193−4.
111. Nechaev S, Gao C, Moreira D, Swiderski P, Jozwiak A, Kowolik CM, et al. Intracellular processing of immunostimulatory CpG−siRNA: Toll-like receptor 9 facilitates siRNA dicing and endosomal escape. *J Control Release* 2013;**170**:307−15.

112. Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, et al. Wnt/beta-catenin pathway: modulating anticancer immune response. *J Hematol Oncol* 2017;**10**:1−12.
113. Matsuda A, Ishiguro K, Yan IK, Patel T. Extracellular vesicle-based therapeutic targeting of β-catenin to modulate anticancer immune responses in hepatocellular cancer. *Hepatol Commun* 2019;**3**:525−41.
114. Wang D, Wang T, Liu J, Yu H, Jiao S, Feng B, et al. Acid-activatable versatile micelleplexes for PD-L1 blockade-enhanced cancer photodynamic immunotherapy. *Nano Lett* 2016;**16**:5503−13.
115. Radtke F, MacDonald HR, Tacchini-Cottier F. Regulation of innate and adaptive immunity by Notch. *Nat Rev Immunol* 2013;**13**:427−35.
116. Li SY, Liu Y, Xu CF, Shen S, Sun R, Du XJ, et al. Restoring antitumor functions of T cells *via* nanoparticle-mediated immune checkpoint modulation. *J Control Release* 2016;**231**:17−28.
117. Wang Y, Xu Z, Guo S, Zhang L, Sharma A, Robertson GP, et al. Intravenous delivery of siRNA targeting CD47 effectively inhibits melanoma tumor growth and lung metastasis. *Mol Ther* 2013;**21**:1919−29.
118. Gao J, Zhang H, Zhou F, Hou B, Chen M, Xie Z, et al. Acid-activatible micelleplex delivering siRNA-PD-L1 for improved cancer immunotherapy of CDK4/6 inhibition. *Chin Chem Lett* 2021;**32**:1929−36.
119. Kim R, Emi M, Tanabe K, Arihiro K. Tumor-driven evolution of immunosuppressive networks during malignant progression. *Cancer Res* 2006;**66**:5527−36.
120. Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, et al. Indoleamine 2,3-dioxygenase pathways of pathogenic inflammation and immune escape in cancer. *Cancer Immunol Immunother* 2014;**63**:721−35.
121. Ye J, Hou B, Chen F, Zhang S, Xiong M, Li T, et al. Bispecific prodrug nanoparticles circumventing multiple immune resistance mechanisms for promoting cancer immunotherapy. *Acta Pharm Sin B* 2022;**12**:2695−709.
122. Wang D, Saga Y, Mizukami H, Sato N, Nonaka H, Fujiwara H, et al. Indoleamine-2,3-dioxygenase, an immunosuppressive enzyme that inhibits natural killer cell function, as a useful target for ovarian cancer therapy. *Int J Oncol* 2012;**40**:929−34.
123. Yen MC, Lin CC, Chen YL, Huang SS, Yang HJ, Chang CP, et al. A novel cancer therapy by skin delivery of indoleamine 2,3-dioxygenase siRNA. *Clin Cancer Res* 2009;**15**:641−9.
124. Song XT, Kabler KE, Shen L, Rollins L, Huang XF, Chen SY. A20 is an antigen presentation attenuator, and its inhibition overcomes regulatory T cell−mediated suppression. *Nat Med* 2008;**14**:258−65.
125. Shi L, Luo K, Xia D, Chen T, Chen G, Jiang Y, et al. DIgR2, dendritic cell-derived immunoglobulin receptor 2, is one representative of a family of IgSF inhibitory receptors and mediates negative regulation of dendritic cell-initiated antigen-specific T-cell responses. *Blood* 2006;**108**:2678−86.
126. Poeck H, Besch R, Maihoefer C, Renn M, Tormo D, Morskaya SS, et al. 5′-Triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma. *Nat Med* 2008;**14**:1256−63.
127. Shen L, Evel-Kabler K, Strube R, Chen SY. Silencing of SOCS1 enhances antigen presentation by dendritic cells and antigen-specific anti-tumor immunity. *Nat Biotechnol* 2004;**22**:1546−53.
128. Li M, Li M, Yang Y, Liu Y, Xie H, Yu Q, et al. Remodeling tumor immune microenvironment *via* targeted blockade of PI3K-γ and CSF-1/CSF-1R pathways in tumor associated macrophages for pancreatic cancer therapy. *J Control Release* 2020;**321**:23−35.
129. Leuschner F, Dutta P, Gorbatov R, Novobrantseva TI, Donahoe JS, Courties G, et al. Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nat Biotechnol* 2011;**29**:1005−10.
130. Hsu YL, Hung JY, Chang WA, Jian SF, Lin YS, Pan YC, et al. Hypoxic lung-cancer-derived extracellular vesicle microRNA-103a increases the oncogenic effects of macrophages by targeting PTEN. *Mol Ther* 2018;**26**:568−81.
131. Wang X, Luo G, Zhang K, Cao J, Huang C, Jiang T, et al. Hypoxic tumor-derived exosomal miR-301a mediates M2 macrophage polarization *via* PTEN/PI3Kγ to promote pancreatic cancer metastasis. *Cancer Res* 2018;**78**:4586−98.
132. Cristino AS, Nourse J, West RA, Sabdia MB, Law SC, Gunawardana J, et al. EBV microRNA-BHRF1-2-5p targets the 3′UTR of immune checkpoint ligands PD-L1 and PD-L2. *Blood* 2019;**134**:2261−70.
133. Parrella P, Barbano R, Pasculli B, Fontana A, Copetti M, Valori VM, et al. Evaluation of microRNA-10b prognostic significance in a prospective cohort of breast cancer patients. *Mol Cancer* 2014;**13**:142.
134. Kotecki N, Opdam F, Robbrecht D, Strijbos M, Kroon K, Janicot M, et al. Phase I/Ib study with INT-1B3, a novel LNP-formulated microRNA (miR-193a-3p mimic) therapeutic for patients with advanced solid cancer. *J Clin Oncol* 2021;**39**:TPS2666.
135. Zhang Y, Liao RB, Hu LL, Tong BX, Hao TF, Wu HJ. The microRNA miR-10b as a potentially promising biomarker to predict the prognosis of cancer patients: a meta-analysis. *Oncotarget* 2017;**8**:104543.
136. Reid G, Kao SC, Pavlakis N, Brahmbhatt H, MacDiarmid J, Clarke S, et al. Clinical development of TargomiRs, a miRNA mimic-based treatment for patients with recurrent thoracic cancer. *Epigenomics* 2016;**8**:1079−85.
137. Foss FM, Querfeld C, Porcu P, Kim YH, Pacheco T, Halwani AS, et al. Phase 1 trial evaluating MRG-106, a synthetic inhibitor of microRNA-155, in patients with cutaneous T-cell lymphoma (CTCL). *J Clin Oncol* 2017;**35**. 7564−7564.
138. Roberts TC, Langer R, Wood MJ. Advances in oligonucleotide drug delivery. *Nat Rev Drug Discov* 2020;**19**:673−94.
139. Eckstein F. Nucleoside phosphorothioates. *J Am Chem Soc* 1970;**92**:4718−23.
140. Eckstein F. Phosphorothioates, essential components of therapeutic oligonucleotides. *Nucleic Acid Ther* 2014;**24**:374−87.
141. Khvorova A, Watts JK. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat Biotechnol* 2017;**35**:238−48.
142. Pandey PR, Young KH, Kumar D, Jain N. RNA-mediated immunotherapy regulating tumor immune microenvironment: next wave of cancer therapeutics. *Mol Cancer* 2022;**21**:1−18.
143. Liang XH, Shen W, Sun H, Migawa MT, Vickers TA, Crooke ST. Translation efficiency of mRNAs is increased by antisense oligonucleotides targeting upstream open reading frames. *Nat Biotechnol* 2016;**34**:875−80.
144. Yoshida T, Morihiro K, Naito Y, Mikami A, Kasahara Y, Inoue T, et al. Identification of nucleobase chemical modifications that reduce the hepatotoxicity of gapmer antisense oligonucleotides. *Nucleic Acids Res* 2022;**50**:7224−34.
145. US Food and Drug Administration. *US Food and Drug Administration FDA approves first drug for spinal muscular atrophy.* Updated March 28, 2018. Available from: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm534611.htm, 2016. [Accessed 15 June 2024].
146. Proia TA, Singh M, Woessner R, Carnevalli L, Bommakanti G, Magiera L, et al. STAT3 antisense oligonucleotide remodels the suppressive tumor microenvironment to enhance immune activation in combination with anti-PD-L1. *Clin Cancer Res* 2020;**26**:6335−49.
147. Renault TT, Chipuk JE. Getting away with murder: how do the BCL-2 family of proteins kill with immunity?. *Ann N Y Acad Sci* 2013;**1285**:59−79.
148. Cheng X, Yu D, Cheng G, Yung BC, Liu Y, Li H, et al. T7 peptide-conjugated lipid nanoparticles for dual modulation of Bcl-2 and Akt-1 in lung and cervical carcinomas. *Mol Pharm* 2018;**15**:4722−32.
149. Kashyap AS, Thelemann T, Klar R, Kallert SM, Festag J, Buchi M, et al. Antisense oligonucleotide targeting CD39 improves anti-tumor T cell immunity. *J Immunother Cancer* 2019;**7**:1−12.
150. Chen Y, Huang S, Guo R, Chen D. Metadherin-mediated mechanisms in human malignancies. *Biomark Med* 2021;**15**:1769−83.
151. Kratschmer C, Levy M. Effect of chemical modifications on aptamer stability in serum. *Nucleic Acid Ther* 2017;**27**:335−44.

152. Zhu G, Chen X. Aptamer-based targeted therapy. *Adv Drug Deliv Rev* 2018;**134**:65−78.
153. Shu D, Zhang L, Bai X, Yu J, Guo P. Stoichiometry of multi-specific immune checkpoint RNA Abs for T cell activation and tumor inhibition using ultra-stable RNA nanoparticles. *Mol Ther Nucleic Acids* 2021;**24**:426−35.
154. Camorani S, Passariello M, Agnello L, Esposito S, Collina F, Cantile M, et al. Aptamer targeted therapy potentiates immune checkpoint blockade in triple-negative breast cancer. *J Exp Clin Cancer Res* 2020;**39**:180.
155. Macdonald J, Denoyer D, Henri J, Jamieson A, Burvenich IJG, Pouliot N, et al. Bifunctional aptamer−doxorubicin conjugate crosses the blood−brain barrier and selectively delivers its payload to EpCAM-positive tumor cells. *Nucleic Acid Ther* 2020;**30**:117−28.
156. Chandra S, Nguyen HM, Wiltz K, Hall N, Chaudhry S, Olverson G, et al. Aptamer-functionalized hybrid nanoparticles to enhance the delivery of doxorubicin into breast cancer cells by silencing P-glycoprotein. *J Cancer Treat Diagn* 2020;**4**:1−13.
157. Shigdar S, Schrand B, Giangrande PH, de Franciscis V. Aptamers: cutting edge of cancer therapies. *Mol Ther* 2021;**29**:2396−411.
158. Wang C, Pan C, Yong H, Wang F, Bo T, Zhao Y, et al. Emerging non-viral vectors for gene delivery. *J Nanobiotechnol* 2023;**21**:272.
159. Wang H, Jiang Y, Peng H, Chen Y, Zhu P, Huang Y. Recent progress in microRNA delivery for cancer therapy by non-viral synthetic vectors. *Adv Drug Deliv Rev* 2015;**81**:142−60.
160. Yan J, Zhang H, Li G, Su J, Wei Y, Xu C. Lipid nanovehicles overcome barriers to systemic RNA delivery: lipid components, fabrication methods, and rational design. *Acta Pharm Sin B* 2024;**14**:579−601.
161. Patel P, Ibrahim NM, Cheng K. The importance of apparent p$K_a$ in the development of nanoparticles encapsulating siRNA and mRNA. *Trends Pharmacol Sci* 2021;**42**:448−60.
162. Albertsen CH, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv Drug Deliv Rev* 2022;**188**:114416.
163. Sarode A, Fan Y, Byrnes AE, Hammel M, Hura GL, Fu Y, et al. Predictive high-throughput screening of PEGylated lipids in oligonucleotide-loaded lipid nanoparticles for neuronal gene silencing. *Nanoscale Adv* 2022;**4**:2107−23.
164. Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, et al. mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability. *Int J Pharm* 2021;**601**:120586.
165. Jia Y, Wang X, Li L, Li F, Zhang J, Liang XJ. Lipid nanoparticles optimized for targeting and release of nucleic acid. *Adv Mater* 2024;**36**:2305300.
166. Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid Nanoparticles—from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano* 2021;**15**:16982−7015.
167. Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ, et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for *in vivo* delivery of siRNA. *Mol Ther Nucleic Acids* 2012;**1**:e37.
168. Karam M, Daoud G. mRNA vaccines: past, present, future. *Asian J Pharml Sci* 2022;**17**:491−522.
169. Kim J, Sunshine JC, Green JJ. Differential polymer structure tunes mechanism of cellular uptake and transfection routes of poly ($\beta$-amino ester) polyplexes in human breast cancer cells. *Bioconjug Chem* 2014;**25**:43−51.
170. Benjaminsen RV, Mattebjerg MA, Henriksen JR, Moghimi SM, Andresen TL. The possible "proton sponge" effect of polyethylenimine (PEI) does not include change in lysosomal pH. *Mol Ther* 2013;**21**:149−57.
171. Taratula O, Garbuzenko OB, Kirkpatrick P, Pandya I, Savla R, Pozharov VP, et al. Surface-engineered targeted PPI dendrimer for efficient intracellular and intratumoral siRNA delivery. *J Control Release* 2009;**140**:284−93.
172. McKinlay CJ, Vargas JR, Blake TR, Hardy JW, Kanada M, Contag CH, et al. Charge-altering releasable transporters (CARTs) for the delivery and release of mRNA in living animals. *Proc Natl Acad Sci U S A* 2017;**114**:E448−56.
173. Al-Dosari MS, Gao X. Nonviral gene delivery: principle, limitations, and recent progress. *AAPS J* 2009;**11**:671−83.
174. Parada N, Romero-Trujillo A, Georges N, Alcayaga-Miranda F. Camouflage strategies for therapeutic exosomes evasion from phagocytosis. *J Adv Res* 2021;**31**:61−74.
175. Zhao L, Gu C, Gan Y, Shao L, Chen H, Zhu H. Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis. *J Control Release* 2020;**318**:1−15.
176. Willms E, Cabañas C, Mäger I, Wood MJ, Vader P. Extracellular vesicle heterogeneity: subpopulations, isolation techniques, and diverse functions in cancer progression. *Front Immunol* 2018;**9**:738.
177. Ahn SH, Ryu SW, Choi H, You S, Park J, Choi C. Manufacturing therapeutic exosomes: from bench to industry. *Mol Cell* 2022;**45**:284−90.
178. Joshi BS, Ortiz D, Zuhorn IS. Converting extracellular vesicles into nanomedicine: loading and unloading of cargo. *Mater Today Nano* 2021;**16**:100148.
179. Cecchin R, Troyer Z, Witwer K, Morris KV. Extracellular vesicles: the next generation in gene therapy delivery. *Mol Ther* 2023;**31**:1225−30.
180. Guo X, Shi H, Zhong W, Xiao H, Liu X, Yu T, et al. Tuning biodegradability and biocompatibility of mesoporous silica nanoparticles by doping strontium. *Ceram Int* 2020;**46**:11762−9.
181. Meng H, Mai WX, Zhang H, Xue M, Xia T, Lin S, et al. Codelivery of an optimal drug/siRNA combination using mesoporous silica nanoparticles to overcome drug resistance in breast cancer *in vitro* and *in vivo*. *ACS Nano* 2013;**7**:994−1005.
182. Na H, Kim M, Park K, Ryoo S, Lee KE, Jeon H, et al. Efficient functional delivery of siRNA using mesoporous silica nanoparticles with ultralarge pores. *Small* 2012;**8**:1752−61.
183. Xiong L, Bi J, Tang Y, Qiao S. Magnetic core−shell silica nanoparticles with large radial mesopores for siRNA delivery. *Small* 2016;**12**:4735−42.
184. Zhou Y, Quan G, Wu Q, Zhang X, Niu B, Wu B, et al. Mesoporous silica nanoparticles for drug and gene delivery. *Acta Pharm Sin B* 2018;**8**:165−77.
185. Zhao Y, Zhao T, Cao Y, Sun J, Zhou Q, Chen H, et al. Temperature-sensitive lipid-coated carbon nanotubes for synergistic photothermal therapy and gene therapy. *ACS Nano* 2021;**15**:6517−29.
186. Habib S, Singh M. Carbon-based Nanomaterials for delivery of small RNA molecules: a focus on potential cancer treatment applications. *Pharm Nanotechnol* 2022;**10**:164−81.
187. Liu Y, Zhao C, Sabirsh A, Ye L, Wu X, Lu H, et al. A novel graphene quantum dot-based mRNA delivery platform. *ChemistryOpen* 2021;**10**:666−71.
188. Demirer GS, Zhang H, Goh NS, Pinals RL, Chang R, Landry MP. Carbon nanocarriers deliver siRNA to intact plant cells for efficient gene knockdown. *Sci Adv* 2020;**6**:eaaz0495.
189. Varkouhi AK, Foillard S, Lammers T, Schiffelers RM, Doris E, Hennink WE, et al. SiRNA delivery with functionalized carbon nanotubes. *Int J Pharm* 2011;**416**:419−25.
190. Liu D, Wang L, Wang Z, Cuschieri A. Magnetoporation and magnetolysis of cancer cells *via* carbon nanotubes induced by rotating magnetic fields. *Nano Lett* 2012;**12**:5117−21.
191. Behzadi S, Serpooshan V, Tao W, Hamaly MA, Alkawareek MY, Dreaden EC, et al. Cellular uptake of nanoparticles: journey inside the cell. *Chem Soc Rev* 2017;**46**:4218−44.
192. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres M del P, Acosta-Torres LS, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol* 2018;**16**:71.
193. Xue C, Hu S, Gao ZH, Wang L, Luo MX, Yu X, et al. Programmably tiling rigidified DNA brick on gold nanoparticle as multi-functional shell for cancer-targeted delivery of siRNAs. *Nat Commun* 2021;**12**:2928.
194. Panday R, Abdalla AME, Yu M, Li X, Ouyang C, Yang G. Functionally modified magnetic nanoparticles for effective siRNA

delivery to prostate cancer cells *in vitro*. *J Biomater Appl* 2020;**34**:952−64.
195. Alsaggar M, Liu D. Physical methods for gene transfer. *Adv Genet* 2015;**89**:1−24.
196. Sharma D, Arora S, Singh J, Layek B. A review of the tortuous path of nonviral gene delivery and recent progress. *Int J Biol Macromol* 2021;**183**:2055−73.
197. Melnick K, Dastmalchi F, Mitchell D, Rahman M, Sayour EJ. Contemporary RNA therapeutics for glioblastoma. *Neuromol Med* 2022;**24**:8−12.
198. Cheng X, Lee RJ. The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery. *Adv Drug Deliv Rev* 2016;**99**:129−37.
199. Smisterová J, Wagenaar A, Stuart MCA, Polushkin E, Brinke G ten, Hulst R, et al. Molecular shape of the cationic lipid controls the structure of cationic lipid/dioleylphosphatidylethanolamine-DNA complexes and the efficiency of gene delivery. *J Biol Chem* 2001;**276**:47615−22.
200. Yu J, Anchordoquy TJ. Synergistic effects of surfactants and sugars on lipoplex stability during freeze-drying and rehydration. *J Pharm Sci* 2009;**98**:3319−28.
201. Hattori Y, Date M, Arai S, Kawano K, Yonemochi E, Maitani Y. Transdermal delivery of small interfering RNA with elastic cationic liposomes in mice. *J Pharm (Lahore)* 2013;**2013**:149695.
202. Zhou C, Mao Y, Sugimoto Y, Zhang Y, Kanthamneni N, Yu B, et al. SPANosomes as delivery vehicles for small interfering RNA (siRNA). *Mol Pharm* 2012;**9**:201−10.
203. Agirre M, Ojeda E, Zarate J, Puras G, Grijalvo S, Eritja R, et al. New insights into gene delivery to human neuronal precursor NT2 cells: a comparative study between lipoplexes, nioplexes, and polyplexes. *Mol Pharm* 2015;**12**:4056−66.
204. Grijalvo S, Puras G, Zárate J, Pons R, Pedraz JL, Eritja R, et al. Nioplexes encapsulated in supramolecular hybrid biohydrogels as versatile delivery platforms for nucleic acids. *RSC Adv* 2016;**6**:39688−99.
205. Kim H, Leal C. Cuboplexes: topologically active siRNA delivery. *ACS Nano* 2015;**9**:10214−26.
206. Sarkar S, Tran N, Soni SK, Nasa Z, Drummond CJ, Conn CE. Cuboplex-mediated nonviral delivery of functional siRNA to Chinese hamster ovary (CHO) cells. *ACS Appl Mater Inter* 2021;**13**:2336−45.
207. Viegas C, Patrício AB, Prata JM, Nadhman A, Chintamaneni PK, Fonte P. Solid lipid nanoparticles *vs.* nanostructured lipid carriers: a comparative review. *Pharmaceutics* 2023;**15**:1593.
208. Piao L, Zhang M, Datta J, Xie X, Su T, Li H, et al. Lipid-based nanoparticle delivery of Pre-miR-107 inhibits the tumorigenicity of head and neck squamous cell carcinoma. *Mol Ther* 2012;**20**:1261−9.
209. Leal C, Bouxsein NF, Ewert KK, Safinya CR. Highly efficient gene silencing activity of siRNA embedded in a nanostructured gyroid cubic lipid matrix. *J Am Chem Soc* 2010;**132**:16841−7.
210. Kim H, Sung J, Chang Y, Alfeche A, Leal C. Microfluidics synthesis of gene silencing cubosomes. *ACS Nano* 2018;**12**:9196−205.
211. Dowdy SF. Overcoming cellular barriers for RNA therapeutics. *Nat Biotechnol* 2017;**35**:222−9.
212. Heyes J, Palmer L, Bremner K, MacLachlan I. Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids. *J Control Release* 2005;**107**:276−87.
213. Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. *Mol Ther* 2017;**25**:1467−75.
214. Jayaraman M, Ansell SM, Mui BL, Tam YK, Chen J, Du X, et al. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing *in vivo*. *Angew Chem Int Ed Engl* 2012;**124**:8657−61.
215. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *New Engl J Med* 2018;**379**:11−21.
216. Akinc A, Maier MA, Manoharan M, Fitzgerald K, Jayaraman M, Barros S, et al. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nat Nanotechnol* 2019;**14**:1084−7.
217. Kim M, Jeong M, Lee G, Lee Y, Park J, Jung H, et al. Novel piperazine-based ionizable lipid nanoparticles allow the repeated dose of mRNA to fibrotic lungs with improved potency and safety. *Bioeng Transl Med* 2023;**8**:e10556.
218. Love KT, Mahon KP, Levins CG, Whitehead KA, Querbes W, Dorkin JR, et al. Lipid-like materials for low-dose, *in vivo* gene silencing. *Proc Natl Acad Sci U S A* 2010;**107**:1864−9.
219. Dong Y, Love KT, Dorkin JR, Sirirungruang S, Zhang Y, Chen D, et al. Lipopeptide nanoparticles for potent and selective siRNA delivery in rodents and nonhuman primates. *Proc Natl Acad Sci U S A* 2014;**111**:3955−60.
220. Miao L, Li L, Huang Y, Delcassian D, Chahal J, Han J, et al. Delivery of mRNA vaccines with heterocyclic lipids increases anti-tumor efficacy by STING-mediated immune cell activation. *Nat Biotechnol* 2019;**37**:1174−85.
221. Akinc A, Querbes W, De S, Qin J, Frank-Kamenetsky M, Jayaprakash KN, et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. *Mol Ther* 2010;**18**:1357−64.
222. Debnath M, Forster J, Ramesh A, Kulkarni A. Protein corona formation on lipid nanoparticles negatively affects the NLRP3 inflammasome activation. *Bioconjug Chem* 2023;**34**:1766−79.
223. He Y, Zheng K, Qin X, Wang S, Li X, Liu H, et al. Intravenous delivery of STING agonists using acid-sensitive polycationic polymer-modified lipid nanoparticles for enhanced tumor immunotherapy. *Acta Pharm Sin B* 2024. Available from: https://doi.org/10.1016/j.apsb.2024.06.004.
224. Dilliard SA, Sun Y, Brown MO, Sung YC, Chatterjee S, Farbiak L, et al. The interplay of quaternary ammonium lipid structure and protein corona on lung-specific mRNA delivery by selective organ targeting (SORT) nanoparticles. *J Control Release* 2023;**361**:361−72.
225. Kong N, Tao W, Ling X, Wang J, Xiao Y, Shi S, et al. Synthetic mRNA nanoparticle-mediated restoration of p53 tumor suppressor sensitizes p53-deficient cancers to mTOR inhibition. *Sci Transl Med* 2019;**11**:eaaw1565.
226. Zhu X, Tao W, Liu D, Wu J, Guo Z, Ji X, et al. Surface De-PEGylation controls nanoparticle-mediated siRNA delivery *in vitro* and *in vivo*. *Theranostics* 2017;**7**:1990.
227. Pathak K, Keshri L, Shah M. Lipid nanocarriers: influence of lipids on product development and pharmacokinetics. *Crit Rev Ther Drug Carrier Syst* 2011;**28**:357−93.
228. Oner E, Kotmakci M, Baird AM, Gray SG, Debelec Butuner B, Bozkurt E, et al. Development of EphA2 siRNA-loaded lipid nanoparticles and combination with a small-molecule histone demethylase inhibitor in prostate cancer cells and tumor spheroids. *J Nanobiotechnol* 2021;**19**:71.
229. Grijalvo S, Puras G, Zárate J, Sainz-Ramos M, Qtaish NA, López T, et al. Cationic niosomes as non-viral vehicles for nucleic acids: challenges and opportunities in gene delivery. *Pharmaceutics* 2019;**11**:50.
230. Hui SW, Langner M, Zhao YL, Ross P, Hurley E, Chan K. The role of helper lipids in cationic liposome-mediated gene transfer. *Biophys J* 1996;**71**:590−9.
231. Paecharoenchai O, Niyomtham N, Ngawhirunpat T, Rojanarata T, Yingyongnarongkul B, Opanasopit P. Cationic niosomes composed of spermine-based cationic lipids mediate high gene transfection efficiency. *J Drug Target* 2012;**20**:783−92.
232. López-Seijas J, Miranda-Balbuena D, Iglesias-Fente A, Sacristán-Santos M, Carballo-Pedrares N, Arufe MC, et al. Development of new non-viral systems for genetic modification of senescent cells. *Mol Ther Nucleic Acids* 2023;**32**:302−17.
233. Wiranowska M, Singh R, Falahat R, Williams E, Johnson JO, Alcantar N. Preferential drug delivery to tumor cells than normal cells using a tunable niosome−chitosan double package nanodelivery system: a novel *in vitro* model. *Cancer Nanotechnol* 2020;**11**:1−20.
234. Kashani-Asadi-Jafari F, Aftab A, Ghaemmaghami S. A machine learning framework for predicting entrapment efficiency in niosomal particles. *Int J Pharm* 2022;**627**:122203.
235. Obeid MA, Elburi A, Young LC, Mullen AB, Tate RJ, Ferro VA. Formulation of nonionic surfactant vesicles (NISV) prepared by

microfluidics for therapeutic delivery of siRNA into cancer cells. *Mol Pharm* 2017;**14**:2450−8.
236. Rittman M, Frischherz M, Burgmann F, Hartley PG, Squires A. Direct visualisation of lipid bilayer cubic phases using atomic force microscopy. *Soft Matter* 2010;**6**:4058−61.
237. Zheng L, Bandara SR, Tan Z, Leal C. Lipid nanoparticle topology regulates endosomal escape and delivery of RNA to the cytoplasm. *Proc Natl Acad Sci U S A* 2023;**120**:e2301067120.
238. Zhen G, Hinton TM, Muir BW, Shi S, Tizard M, McLean KM, et al. Glycerol monooleate-based nanocarriers for siRNA delivery *in vitro*. *Mol Pharm* 2012;**9**:2450−7.
239. Lim JM, Bertrand N, Valencia PM, Rhee M, Langer R, Jon S, et al. Parallel microfluidic synthesis of size-tunable polymeric nanoparticles using 3D flow focusing towards *in vivo* study. *Nanomed Nanotechnol Biol Med* 2014;**10**:401−9.
240. Shepherd SJ, Warzecha CC, Yadavali S, El-Mayta R, Alameh MG, Wang L, et al. Scalable mRNA and siRNA lipid nanoparticle production using a parallelized microfluidic device. *Nano Lett* 2021;**21**:5671−80.
241. Lopes C, Cristóvão J, Silvério V, Lino PR, Fonte P. Microfluidic production of mRNA-loaded lipid nanoparticles for vaccine applications. *Expert Opin Drug Deliv* 2022;**19**:1381−95.
242. Shepherd SJ, Han X, Mukalel AJ, El-Mayta R, Thatte AS, Wu J, et al. Throughput-scalable manufacturing of SARS-CoV-2 mRNA lipid nanoparticle vaccines. *Proc Natl Acad Sci U S A* 2023;**120**:e2303567120.
243. Tian F, Cai L, Liu C, Sun J. Microfluidic technologies for nanoparticle formation. *Lab Chip* 2022;**22**:512−29.
244. McKenzie RE, Minnell JJ, Ganley M, Painter GF, Draper SL. mRNA synthesis and encapsulation in ionizable lipid nanoparticles. *Curr Protoc* 2023;**3**:e898.
245. Maeki M, Kimura N, Okada Y, Shimizu K, Shibata K, Miyazaki Y, et al. Understanding the effects of ethanol on the liposome bilayer structure using microfluidic-based time-resolved small-angle X-ray scattering and molecular dynamics simulations. *Nanoscale Adv* 2024;**6**:2166−76.
246. Meng C, Chen Z, Li G, Welte T, Shen H. Nanoplatforms for mRNA therapeutics. *Adv Ther* 2021;**4**:2000099.
247. Xia Y, Fu S, Ma Q, Liu Y, Zhang N. Application of nano-delivery systems in lymph nodes for tumor immunotherapy. *Nanomicro Lett* 2023;**15**:145.
248. Tang Z, He C, Tian H, Ding J, Hsiao BS, Chu B, et al. Polymeric nanostructured materials for biomedical applications. *Prog Polym Sci* 2016;**60**:86−128.
249. Kandil R, Merkel OM. Recent progress of polymeric nanogels for gene delivery. *Curr Opin Colloid Interf Sci* 2019;**39**:11−23.
250. Kuperkar K, Patel D, Atanase LI, Bahadur P. Amphiphilic block copolymers: their structures, and self-assembly to polymeric micelles and polymersomes as drug delivery vehicles. *Polym* 2022;**14**:4702.
251. Lin W, Kim D. pH-Sensitive micelles with cross-linked cores formed from polyaspartamide derivatives for drug delivery. *Langmuir* 2011;**27**:12090−7.
252. Pathak A, Patnaik S, Gupta KC. Recent trends in non-viral vector-mediated gene delivery. *Biotechnol J* 2009;**4**:1559−72.
253. Marras AE, Ting JM, Stevens KC, Tirrell MV. Advances in the structural design of polyelectrolyte complex micelles. *J Phys Chem B* 2021;**125**:7076−89.
254. Neu M, Fischer D, Kissel T. Recent advances in rational gene transfer vector design based on poly (ethylene imine) and its derivatives. *J Gene Med* 2005;**7**:992−1009.
255. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: polyethylenimine. *Proc Natl Acad Sci U S A* 1995;**92**:7297−301.
256. Johler SM, Rejman J, Guan S, Rosenecker J. Nebulisation of IVT mRNA complexes for intrapulmonary administration. *PLoS One* 2015;**10**:e0137504.
257. Cortez-Jugo C, Masoumi S, Chan PP, Friend J, Yeo L. Nebulization of siRNA for inhalation therapy based on a microfluidic surface acoustic wave platform. *Ultrason Sonochem* 2022;**88**:106088.
258. Choi YJ, Kang SJ, Kim YJ, Lim Y, Chung HW. Comparative studies on the genotoxicity and cytotoxicity of polymeric gene carriers polyethylenimine (PEI) and polyamidoamine (PAMAM) dendrimer in Jurkat T-cells. *Drug Chem Toxicol* 2010;**33**:357−66.
259. Zhao M, Li M, Zhang Z, Gong T, Sun X. Induction of HIV-1 gag specific immune responses by cationic micelles mediated delivery of gag mRNA. *Drug Deliv* 2016;**23**:2596−607.
260. Chiper M, Tounsi N, Kole R, Kichler A, Zuber G. Self-aggregating 1.8 kDa polyethylenimines with dissolution switch at endosomal acidic pH are delivery carriers for plasmid DNA, mRNA, siRNA and exon-skipping oligonucleotides. *J Control Release* 2017;**246**:60−70.
261. Tripathi SK, Yadav S, Gupta KC, Kumar P. Synthesis and evaluation of *N*-(2,3-dihydroxypropyl)-PEIs as efficient vectors for nucleic acids. *Mol Biosyst* 2012;**8**:1426−34.
262. Dong C, Wang Y, Zhu W, Ma Y, Kim J, Wei L, et al. Polycationic HA/CpG nanoparticles induce cross-protective influenza immunity in mice. *ACS Appl Mater Inter* 2022;**14**:6331−42.
263. Neu M, Fischer D, Kissel T. Recent advances in rational gene transfer vector design based on poly(ethylene imine) and its derivatives. *J Gene Med* 2005;**7**:992−1009.
264. Kang HC, Kang HJ, Bae YH. A reducible polycationic gene vector derived from thiolated low molecular weight branched polyethyleneimine linked by 2-iminothiolane. *Biomaterials* 2011;**32**:1193−203.
265. Wang Y, Zheng M, Meng F, Zhang J, Peng R, Zhong Z. Branched polyethylenimine derivatives with reductively cleavable periphery for safe and efficient *in vitro* gene transfer. *Biomacromolecules* 2011;**12**:1032−40.
266. Tan L, Zheng T, Li M, Zhong X, Tang Y, Qin M, et al. Optimization of an mRNA vaccine assisted with cyclodextrin-polyethyleneimine conjugates. *Drug Deliv Transl Res* 2020;**10**:678−89.
267. Adelnia H, Tran HDN, Little PJ, Blakey I, Ta HT. Poly(aspartic acid) in biomedical applications: from polymerization, modification, properties, degradation, and biocompatibility to applications. *ACS Biomater Sci Eng* 2021;**7**:2083−105.
268. Miyata K, Oba M, Nakanishi M, Fukushima S, Yamasaki Y, Koyama H, et al. Polyplexes from poly (aspartamide) bearing 1,2-diaminoethane side chains induce pH-selective, endosomal membrane destabilization with amplified transfection and negligible cytotoxicity. *J Am Chem Soc* 2008;**130**:16287−94.
269. Uchida H, Itaka K, Uchida S, Ishii T, Suma T, Miyata K, et al. Synthetic polyamines to regulate mRNA translation through the preservative binding of eukaryotic initiation factor 4E to the cap structure. *J Am Chem Soc* 2016;**138**:1478−81.
270. Gao H, Takemoto H, Chen Q, Naito M, Uchida H, Liu X, et al. Regulated protonation of polyaspartamide derivatives bearing repeated aminoethylene side chains for efficient intracellular siRNA delivery with minimal cytotoxicity. *Chem Commun* 2015;**51**:3158−61.
271. Lopukhov AV, Yang Z, Haney MJ, Bronich TK, Sokolsky-Papkov M, Batrakova EV, et al. Mannosylated cationic copolymers for gene delivery to macrophages. *Macromol Biosci* 2021;**21**:2000371.
272. Truong NP, Gu W, Prasadam I, Jia Z, Crawford R, Xiao Y, et al. An influenza virus-inspired polymer system for the timed release of siRNA. *Nat Commun* 2013;**4**:1902.
273. Ulkoski D, Munson MJ, Jacobson ME, Palmer CR, Carson CS, Sabirsh A, et al. High-throughput automation of endosomolytic polymers for mRNA delivery. *ACS Appl Bio Mater* 2021;**4**:1640−54.
274. Pafiti KS, Patrickios CS, Georgiou TK, Yamasaki EN, Mastroyiannopoulos NP, Phylactou LA. Cationic star polymer siRNA transfectants interconnected with a piperazine-based cationic crosslinker. *Eur Polym J* 2012;**48**:1422−30.
275. Zhu C, Jung S, Luo S, Meng F, Zhu X, Park TG, et al. Co-delivery of siRNA and paclitaxel into cancer cells by biodegradable cationic micelles based on PDMAEMA−PCL−PDMAEMA triblock copolymers. *Biomaterials* 2010;**31**:2408−16.

276. You YZ, Manickam DS, Zhou QH, Oupický D. Reducible poly (2-dimethylaminoethyl methacrylate): synthesis, cytotoxicity, and gene delivery activity. *J Control Release* 2007;**122**:217−25.
277. Uchida H, Itaka K, Nomoto T, Ishii T, Suma T, Ikegami M, et al. Modulated protonation of side chain aminoethylene repeats in *N*-substituted polyaspartamides promotes mRNA transfection. *J Am Chem Soc* 2014;**136**:12396−405.
278. Samir A, Ashour FH, Hakim AA, Bassyouni M. Recent advances in biodegradable polymers for sustainable applications. *Npj Mater Degrad* 2022;**6**:68.
279. Jensen DK, Jensen LB, Koocheki S, Bengtson L, Cun D, Nielsen HM, et al. Design of an inhalable dry powder formulation of DOTAP-modified PLGA nanoparticles loaded with siRNA. *J Control Release* 2012;**157**:141−8.
280. Zhao ZX, Gao SY, Wang JC, Chen CJ, Zhao EY, Hou WJ, et al. Self-assembly nanomicelles based on cationic mPEG-PLA-*b*-polyarginine (R15) triblock copolymer for siRNA delivery. *Biomaterials* 2012;**33**:6793−807.
281. Li Y, Zhang J, Wang B, Shen Y, Ouahab A. Co-delivery of siRNA and hypericin into cancer cells by hyaluronic acid modified PLGA-PEI nanoparticles. *Drug Dev Ind Pharm* 2016;**42**:737−46.
282. Elmowafy EM, Tiboni M, Soliman ME. Biocompatibility, biodegradation and biomedical applications of poly (lactic acid)/poly(lactic-*co*-glycolic acid) micro and nanoparticles. *J Pharm Investig* 2019;**49**:347−80.
283. Yuan X, Shah BA, Kotadia NK, Li J, Gu H, Wu Z. The development and mechanism studies of cationic chitosan-modified biodegradable PLGA nanoparticles for efficient siRNA drug delivery. *Pharm Res (N Y)* 2010;**27**:1285−95.
284. Benner NL, McClellan RL, Turlington CR, Haabeth OA, Waymouth RM, Wender PA. Oligo (serine ester) charge-altering releasable transporters: organocatalytic ring-opening polymerization and their use for *in vitro* and *in vivo* mRNA delivery. *J Am Chem Soc* 2019;**141**:8416−21.
285. Tanaka H, Takahashi T, Konishi M, Takata N, Gomi M, Shirane D, et al. Self-degradable lipid-like materials based on "hydrolysis accelerated by the intra-particle enrichment of reactant (HyPER)" for messenger RNA delivery. *Adv Funct Mater* 2020;**30**:1910575.
286. McKinlay CJ, Benner NL, Haabeth OA, Waymouth RM, Wender PA. Enhanced mRNA delivery into lymphocytes enabled by lipid-varied libraries of charge-altering releasable transporters. *Proc Natl Acad Sci U S A* 2018;**115**:E5859−66.
287. Benner NL, McClellan RL, Turlington CR, Haabeth OA, Waymouth RM, Wender PA. Oligo (serine ester) charge-altering releasable transporters: organocatalytic ring-opening polymerization and their use for *in vitro* and *in vivo* mRNA delivery. *J Am Chem Soc* 2019;**141**:8416−21.
288. Blake TR, Haabeth OA, Sallets A, McClellan RL, Del Castillo TJ, Vilches-Moure JG, et al. Lysine-derived charge-altering releasable transporters: targeted delivery of mRNA and siRNA to the lungs. *Bioconjug Chem* 2023;**34**:673−85.
289. Parelkar SS, Chan-Seng D, Emrick T. Reconfiguring polylysine architectures for controlling polyplex binding and non-viral transfection. *Biomaterials* 2011;**32**:2432−44.
290. Won YW, Kim HA, Lee M, Kim YH. Reducible poly (oligo-D-arginine) for enhanced gene expression in mouse lung by intratracheal injection. *Mol Ther* 2010;**18**:734−42.
291. Shi M, Zhang J, Huang Z, Chen Y, Pan S, Hu H, et al. Stimuli-responsive release and efficient siRNA delivery in non-small cell lung cancer by a poly(L-histidine)-based multifunctional nanoplatform. *J Mater Chem B* 2020;**8**:1616−28.
292. Jiang X, Abedi K, Shi J. Polymeric nanoparticles for RNA delivery. Reference module in materials science and materials engineering. In: Yin Y, Lu Y, Xia Y, editors. *Encyclopedia of nanomaterials*. 1st ed. Amsterdam: Elsevier; 2021. p. 555−73.
293. Hoerr I, Obst R, Rammensee H, Jung G. In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies. *Eur J Immunol* 2000;**30**:1−7.
294. Zhang X, Men K, Zhang Y, Zhang R, Yang L, Duan X. Local and systemic delivery of mRNA encoding survivin-T34A by lipoplex for efficient colon cancer gene therapy. *Int J Nanomed* 2019;**14**:2733−51.
295. Mäe M, Langel Ü. Cell-penetrating peptides as vectors for peptide, protein and oligonucleotide delivery. *Curr Opin Pharmacol* 2006;**6**:509−14.
296. Tashima T. Intelligent substance delivery into cells using cell-penetrating peptides. *Bioorg Med Chem Lett* 2017;**27**:121−30.
297. van den Brand D, Gorris MA, van Asbeck AH, Palmen E, Ebisch I, Dolstra H, et al. Peptide-mediated delivery of therapeutic mRNA in ovarian cancer. *Eur J Pharm Biopharm* 2019;**141**:180−90.
298. Li W, Nicol F, Szoka FC. GALA: a designed synthetic pH-responsive amphipathic peptide with applications in drug and gene delivery. *Adv Drug Deliv Rev* 2004;**56**:967−85.
299. Lou B, De Koker S, Lau CYJ, Hennink WE, Mastrobattista E. mRNA polyplexes with post-conjugated GALA peptides efficiently target, transfect, and activate antigen presenting cells. *Bioconjug Chem* 2018;**30**:461−75.
300. Wang J, Li B, Qiu L, Qiao X, Yang H. Dendrimer-based drug delivery systems: history, challenges, and latest developments. *J Biol Eng* 2022;**16**:18.
301. Yang J, Zhang Q, Chang H, Cheng Y. Surface-engineered dendrimers in gene delivery. *Chem Rev* 2015;**115**:5274−300.
302. Tietze S, Schau I, Michen S, Ennen F, Janke A, Schackert G, et al. A poly(propyleneimine) dendrimer-based polyplex-system for single-chain antibody-mediated targeted delivery and cellular uptake of SiRNA. *Small* 2017;**13**:1700072.
303. Ulkoski D, Bak A, Wilson JT, Krishnamurthy VR. Recent advances in polymeric materials for the delivery of RNA therapeutics. *Expert Opin Drug Deliv* 2019;**16**:1149−67.
304. Roberts JC, Bhalgat MK, Zera RT. Preliminary biological evaluation of polyamidoamine (PAMAM) StarburstTM dendrimers. *J Biome Mater Res* 1996;**30**:53−65.
305. Shah N, Steptoe RJ, Parekh HS. Low-generation asymmetric dendrimers exhibit minimal toxicity and effectively complex DNA. *J Pept Sci* 2011;**17**:470−8.
306. Liu H, Wang H, Yang W, Cheng Y. Disulfide cross-linked low generation dendrimers with high gene transfection efficacy, low cytotoxicity, and low cost. *J Am Chem Soc* 2012;**134**:17680−7.
307. Kretzmann JA, Ho D, Evans CW, Plani-Lam JH, Garcia-Bloj B, Mohamed AE, et al. Synthetically controlling dendrimer flexibility improves delivery of large plasmid DNA. *Chem Sci* 2017;**8**:2923−30.
308. Wang M, Liu H, Li L, Cheng Y. A fluorinated dendrimer achieves excellent gene transfection efficacy at extremely low nitrogen to phosphorus ratios. *Nat Commun* 2014;**5**:3053.
309. Tzeng SY, Hung BP, Grayson WL, Green JJ. Cystamine-terminated poly (beta-amino ester) s for siRNA delivery to human mesenchymal stem cells and enhancement of osteogenic differentiation. *Biomaterials* 2012;**33**:8142−51.
310. Karlsson J, Rhodes KR, Green JJ, Tzeng SY. Poly(beta-amino ester)s as gene delivery vehicles: challenges and opportunities. *Expert Opin Drug Deliv* 2020;**17**:1395−410.
311. Gao Y, Huang JY, O'Keeffe Ahern J, Cutlar L, Zhou D, Lin FH, et al. Highly branched poly (*β*-amino esters) for non-viral gene delivery: high transfection efficiency and low toxicity achieved by increasing molecular weight. *Biomacromolecules* 2016;**17**:3640−7.
312. Fornaguera C, Guerra-Rebollo M, Angel Lazaro M, Castells-Sala C, Meca-Cortés O, Ramos-Pérez V, et al. mRNA delivery system for targeting antigen-presenting cells *in vivo*. *Adv Healthc Mater* 2018;**7**:1800335.
313. Capasso Palmiero U, Kaczmarek JC, Fenton OS, Anderson DG. Poly(*β*-amino ester)-*co*-poly(caprolactone) terpolymers as nonviral

vectors for mRNA delivery *in vitro* and *in vivo*. *Adv Healthc Mater* 2018;**7**:1800249.
314. Parayath N, Stephan S, Koehne A, Nelson P, Stephan M. *In vitro*-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells *in vivo*. *Nat Commun* 2020;**11**:6080.
315. Smith TT, Stephan SB, Moffett HF, McKnight LE, Ji W, Reiman D, et al. *In situ* programming of leukaemia-specific T cells using synthetic DNA nanocarriers. *Nat Nanotechnol* 2017;**12**:813−20.
316. Patel AK, Kaczmarek JC, Bose S, Kauffman KJ, Mir F, Heartlein MW, et al. Inhaled nanoformulated mRNA polyplexes for protein production in lung epithelium. *Adv Mater* 2019;**31**:1805116.
317. Rui Y, Varanasi M, Mendes S, Yamagata HM, Wilson DR, Green JJ. Poly(beta-amino ester) nanoparticles enable nonviral delivery of CRISPR-Cas9 plasmids for gene knockout and gene deletion. *Mol Ther Nucleic Acids* 2020;**20**:661−72.
318. Stenzel MH. Glycopolymers for drug delivery: opportunities and challenges. *Macromolecules* 2022;**55**:4867−90.
319. Barclay TG, Day CM, Petrovsky N, Garg S. Review of polysaccharide particle-based functional drug delivery. *Carbohydr Polym* 2019;**221**:94−112.
320. Liu X, Howard KA, Dong M, Andersen MØ, Rahbek UL, Johnsen MG, et al. The influence of polymeric properties on chitosan/siRNA nanoparticle formulation and gene silencing. *Biomaterials* 2007;**28**:1280−8.
321. Malmo J, Sørgård H, Vårum KM, Strand SP. siRNA delivery with chitosan nanoparticles: molecular properties favoring efficient gene silencing. *J Controlled Release* 2012;**158**:261−8.
322. Cao Y, Tan YF, Wong YS, Aminuddin M, Ramya B, Liew MWJ, et al. Designing siRNA/chitosan-methacrylate complex nanolipogel for prolonged gene silencing effects. *Sci Rep* 2022;**12**:3527.
323. Muddineti OS, Shah A, Rompicharla SVK, Ghosh B, Biswas S. Cholesterol-grafted chitosan micelles as a nanocarrier system for drug-siRNA co-delivery to the lung cancer cells. *Int J Biol Macromol* 2018;**118**:857−63.
324. Sadio A, Gustafsson JK, Pereira B, Gomes CP, Hansson GC, David L, et al. Modified-chitosan/siRNA nanoparticles downregulate cellular CDX2 expression and cross the gastric mucus barrier. *PLoS One* 2014;**9**:e99449.
325. Kogan G, Šoltés L, Stern R, Gemeiner P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol Lett* 2007;**29**:17−25.
326. Surace C, Arpicco S, Dufaÿ-Wojcicki A, Marsaud V, Bouclier C, Clay D, et al. Lipoplexes targeting the CD44 hyaluronic acid receptor for efficient transfection of breast cancer cells. *Mol Pharm* 2009;**6**:1062−73.
327. Mattheolabakis G, Milane L, Singh A, Amiji MM. Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. *J Drug Target* 2015;**23**:605−18.
328. Castillo Cruz B, Flores Colon M, Rabelo Fernandez RJ, Vivas-Mejia PE, Barletta GL. A fresh look at the potential of cyclodextrins for improving the delivery of siRNA encapsulated in liposome nanocarriers. *ACS Omega* 2022;**7**:3731−7.
329. Wang Y, Shim MS, Levinson NS, Sung H, Xia Y. Stimuli-responsive materials for controlled release of theranostic agents. *Adv Funct Mater* 2014;**24**:4206−20.
330. Fang Z, Zhang X, Huang H, Wu J. Exosome based miRNA delivery strategy for disease treatment. *Chin Chem Lett* 2022;**33**:1693−704.
331. Patel MM, Patel BM. Crossing the blood−brain barrier: recent advances in drug delivery to the brain. *CNS Drugs* 2017;**31**:109−33.
332. Ahn SH, Ryu SW, Choi H, You S, Park J, Choi C. Manufacturing therapeutic exosomes: from bench to industry. *Mol Cell* 2022;**45**:284.
333. Fu S, Wang Y, Xia X, Zheng JC. Exosome engineering: current progress in cargo loading and targeted delivery. *NanoImpact* 2020;**20**:100261.
334. Zomer A, Vendrig T, Hopmans ES, van Eijndhoven M, Middeldorp JM, Pegtel DM. Exosomes: fit to deliver small RNA. *Commun Integr Biol* 2010;**3**:447−50.
335. Charoenviriyakul C, Takahashi Y, Morishita M, Matsumoto A, Nishikawa M, Takakura Y. Cell type-specific and common characteristics of exosomes derived from mouse cell lines: yield, physicochemical properties, and pharmacokinetics. *Eur J Pharm Sci* 2017;**96**:316−22.
336. Wu JY, Li YJ, Hu XB, Huang S, Xiang DX. Preservation of small extracellular vesicles for functional analysis and therapeutic applications: a comparative evaluation of storage conditions. *Drug Deliv* 2021;**28**:162−70.
337. Jeyaram A, Jay SM. Preservation and storage stability of extracellular vesicles for therapeutic applications. *AAPS J* 2018;**20**:1−7.
338. Kumar S, Michael IJ, Park J, Granick S, Cho Y. Cloaked exosomes: biocompatible, durable, and degradable encapsulation. *Small* 2018;**14**:1802052.
339. Wang C, Kimura K, Li J, Richardson JJ, Naito M, Miyata K, et al. Polydopamine-mediated surface functionalization of exosomes. *ChemNanoMat* 2021;**7**:592−5.
340. Amondarain M, Gallego I, Puras G, Saenz-del-Burgo L, Luzzani C, Pedraz JL. The role of microfluidics and 3D-bioprinting in the future of exosome therapy. *Trends Biotechnol* 2023;**41**:1343−59.
341. Xiong L. *Mesoporous silica nanocomposites for drug and gene delivery*. Dissertation. University of Adelaide; 2016. p. 24.
342. Wu M, Meng Q, Chen Y, Zhang L, Li M, Cai X, et al. Large pore-sized hollow mesoporous organosilica for redox-responsive gene delivery and synergistic cancer chemotherapy. *Adv Mater* 2016;**28**:1963−9.
343. Wang Y, Song H, Yu M, Xu C, Liu Y, Tang J, et al. Room temperature synthesis of dendritic mesoporous silica nanoparticles with small sizes and enhanced mRNA delivery performance. *J Mater Chem B* 2018;**6**:4089−95.
344. Wang Y, Song H, Liu C, Zhang Y, Kong Y, Tang J, et al. Confined growth of ZIF-8 in dendritic mesoporous organosilica nanoparticles as bioregulators for enhanced mRNA delivery *in vivo*. *Natl Sci Rev* 2021;**8**:nwaa268.
345. Xiong L, Qiao SZ. A mesoporous organosilica nano-bowl with high DNA loading capacity−a potential gene delivery carrier. *Nanoscale* 2016;**8**:17446−50.
346. Minami K, Okamoto K, Harano K, Noiri E, Nakamura E. Hierarchical assembly of siRNA with tetraamino fullerene in physiological conditions for efficient internalization into cells and knockdown. *ACS Appl Mater Inter* 2018;**10**:19347−54.
347. Liu Z, Winters M, Holodniy M, Dai H. siRNA delivery into human T cells and primary cells with carbon-nanotube transporters. *Angew Chem Int Ed Engl* 2007;**46**:2023−7.
348. Wang L, Wang X, Bhirde A, Cao J, Zeng Y, Huang X, et al. Carbon dots based two-photon visible nanocarriers for safe and highly efficient delivery of siRNA and DNA. *Adv Healthc Mater* 2014;**3**:1203.
349. Wu YF, Wu HC, Kuan CH, Lin CJ, Wang LW, Chang CW, et al. Multi-functionalized carbon dots as theranostic nanoagent for gene delivery in lung cancer therapy. *Sci Rep* 2016;**6**:21170.
350. Shu M, Gao F, Yu C, Zeng M, He G, Wu Y, et al. Dual-targeted therapy in HER2-positive breast cancer cells with the combination of carbon dots/HER3 siRNA and trastuzumab. *Nanotechnology* 2020;**31**:335102.
351. Chen P, He X, Hu Y, Tian XL, Yu XQ, Zhang J. Spleen-targeted mRNA delivery by amphiphilic carbon dots for tumor immunotherapy. *ACS Appl Mater Inter* 2023;**15**:19937−50.
352. Siu KS, Chen D, Zheng X, Zhang X, Johnston N, Liu Y, et al. Non-covalently functionalized single-walled carbon nanotube for topical siRNA delivery into melanoma. *Biomaterials* 2014;**35**:3435−42.
353. Claveau S, Kindermann M, Papine A, Díaz-Riascos ZV, Delen X, Georges P, et al. Harnessing subcellular-resolved organ distribution of cationic copolymer-functionalized fluorescent nanodiamonds for optimal delivery of active siRNA to a xenografted tumor in mice. *Nanoscale* 2021;**13**:9280−92.
354. Reina G, Zhao L, Bianco A, Komatsu N. Chemical functionalization of nanodiamonds: opportunities and challenges ahead. *Angew Chem Int Ed Engl* 2019;**58**:17918−29.

355. Claveau S, Nehlig É, Garcia-Argote S, Feuillastre S, Pieters G, Girard HA, et al. Delivery of siRNA to Ewing sarcoma tumor xenografted on mice, using hydrogenated detonation nanodiamonds: treatment efficacy and tissue distribution. *Nanomaterials* 2020;**10**:553.
356. Liao WS, Ho Y, Lin YW, Raj EN, Liu KK, Chen C, et al. Targeting EGFR of triple-negative breast cancer enhances the therapeutic efficacy of paclitaxel-and cetuximab-conjugated nanodiamond nanocomposite. *Acta Biomater* 2019;**86**:395−405.
357. Xu J, Gu M, Hooi L, Toh TB, Thng DKH, Lim JJ, et al. Enhanced penetrative siRNA delivery by a nanodiamond drug delivery platform against hepatocellular carcinoma 3D models. *Nanoscale* 2021;**13**:16131−45.
358. Lee JS, Green JJ, Love KT, Sunshine J, Langer R, Anderson DG. Gold, poly ($\beta$-amino ester) nanoparticles for small interfering RNA delivery. *Nano Lett* 2009;**9**:2402−6.
359. Scherer F, Anton M, Schillinger U, Henke J, Bergemann C, Krüger A, et al. Magnetofection: enhancing and targeting gene delivery by magnetic force *in vitro* and *in vivo*. *Gene Ther* 2002;**9**:102−9.
360. Houseley J, Tollervey D. The many pathways of RNA degradation. *Cell* 2009;**136**:763−76.
361. Spadea A, Jackman M, Cui L, Pereira S, Lawrence MJ, Campbell RA, et al. Nucleic acid-loaded lipid nanoparticle interactions with model endosomal membranes. *ACS Appl Mater Inter* 2022;**14**:30371−84.
362. Ramirez F, Zambrano A, Hennis R, Holland N, Lakshmanaswamy R, Chacon J. Sending a message: use of mRNA vaccines to target the tumor immune microenvironment. *Vaccines* 2023;**11**:1465.
363. Paul A, Muralidharan A, Biswas A, Kamath BV, Joseph A, Alex AT. siRNA therapeutics and its challenges: recent advances in effective delivery for cancer therapy. *OpenNano* 2022;**7**:100063.
364. Sun S, Hao H, Yang G, Zhang Y, Fu Y. Immunotherapy with CAR-modified T cells: toxicities and overcoming strategies. *J Immunol Res* 2018;**2018**:2386187.
365. August A, Brito L, Paris R, Zaks T. Clinical development of mRNA vaccines: challenges and opportunities. *Curr Top Microbiol Immunol* 2022;**440**:167−86.
366. Husain SR, Han J, Au P, Shannon K, Puri RK. Gene therapy for cancer: regulatory considerations for approval. *Cancer Gene Ther* 2015;**22**:554.
367. Martin A. *Optimisation of ultrasound-mediated delivery of mRNA to mammalian cells[Dissertation]*. University of Oxford; 2022. p. 1−35.
368. Emami Nejad A, Najafgholian S, Rostami A, Sistani A, Shojaeifar S, Esparvarinha M, et al. The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment. *Cancer Cell Int* 2021;**21**:1−26.
369. Chen WL, Wang CC, Lin YJ, Wu CP, Hsieh CH. Cycling hypoxia induces chemoresistance through the activation of reactive oxygen species-mediated B-cell lymphoma extra-long pathway in glioblastoma multiforme. *J Transl Med* 2015;**13**:1−13.
370. Jing X, Yang F, Shao C, Wei K, Xie M, Shen H, et al. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Mol Cancer* 2019;**18**:1−15.
371. Poon E, Harris AL, Ashcroft M. Targeting the hypoxia-inducible factor (HIF) pathway in cancer. *Expert Rev Mol Med* 2009;**11**:e26.
372. Choudhry H, Harris AL. Advances in hypoxia-inducible factor biology. *Cell Metab* 2018;**27**:281−98.
373. Hendrickson MD, Poyton RO. Crosstalk between nitric oxide and hypoxia-inducible factor signaling pathways: an update. *Res Rep Biochem* 2015;**5**:147−61.
374. Chowdhury R, Godoy LC, Thiantanawat A, Trudel LJ, Deen WM, Wogan GN. Nitric oxide produced endogenously is responsible for hypoxia-induced HIF-1$\alpha$ stabilization in colon carcinoma cells. *Chem Res Toxicol* 2012;**25**:2194−202.
375. Infantino V, Santarsiero A, Convertini P, Todisco S, Iacobazzi V. Cancer cell metabolism in hypoxia: role of HIF-1 as key regulator and therapeutic target. *Int J Mol Sci* 2021;**22**:5703.
376. Brahimi-Horn MC, Chiche J, Pouysségur J. Hypoxia and cancer. *J Mol Med* 2007;**85**:1301−7.
377. Le A, Lane AN, Hamaker M, Bose S, Gouw A, Barbi J, et al. Glucose-independent glutamine metabolism *via* TCA cycling for proliferation and survival in B cells. *Cell Metab* 2012;**15**:110−21.
378. Lane LA, Qian X, Smith AM, Nie S. Physical chemistry of nanomedicine: understanding the complex behaviors of nanoparticles *in vivo*. *Ann Rev Phys Chem* 2015;**66**:521−47.
379. Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, et al. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. *Integr Biol* 2015;**7**:1120−34.
380. Pan Y, Yu Y, Wang X, Zhang T. Tumor-associated macrophages in tumor immunity. *Front Immunol* 2020;**11**:583084.
381. Chen S, Saeed AF, Liu Q, Jiang Q, Xu H, Xiao GG, et al. Macrophages in immunoregulation and therapeutics. *Signal Transduct Target Ther* 2023;**8**:207.
382. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat Med* 2018;**24**:1277−89.
383. Chakravarthy A, Khan L, Bensler NP, Bose P, De Carvalho DD. TGF-$\beta$-associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure. *Nat Commun* 2018;**9**:4692.
384. Bunting MD, Vyas M, Requesens M, Langenbucher A, Schiferle EB, Manguso RT, et al. Extracellular matrix proteins regulate NK cell function in peripheral tissues. *Sci Adv* 2022;**8**:eabk3327.
385. Rygiel TP, Stolte EH, de Ruiter T, van de Weijer ML, Meyaard L. Tumor-expressed collagens can modulate immune cell function through the inhibitory collagen receptor LAIR-1. *Mol Immunol* 2011;**49**:402−6.
386. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet AL, et al. VEGF-A modulates expression of inhibitory checkpoints on $CD8^+$ T cells in tumors. *J Exp Med* 2015;**212**:139−48.
387. Cho H, Matsumoto S, Fujita Y, Kuroda A, Menju T, Sonobe M, et al. Trametinib plus 4-methylumbelliferone exhibits antitumor effects by ERK blockade and CD44 downregulation and affects PD-1 and PD-L1 in malignant pleural mesothelioma. *J Thorac Oncol* 2017;**12**:477−90.
388. Huang J, Zhang L, Wan D, Zhou L, Zheng S, Lin S, et al. Extracellular matrix and its therapeutic potential for cancer treatment. *Signal Transduct Target Ther* 2021;**6**:1−24.
389. Bazak R, Houri M, El Achy S, Hussein W, Refaat T. Passive targeting of nanoparticles to cancer: a comprehensive review of the literature. *Mol Clin Oncol* 2014;**2**:904−8.
390. Kalyane D, Raval N, Maheshwari R, Tambe V, Kalia K, Tekade RK. Employment of enhanced permeability and retention effect (EPR): nanoparticle-based precision tools for targeting of therapeutic and diagnostic agent in cancer. *Mater Sci Eng C* 2019;**98**:1252−76.
391. Stylianopoulos T, Munn LL, Jain RK. Reengineering the physical microenvironment of tumors to improve drug delivery and efficacy: from mathematical modeling to bench to bedside. *Trends Cancer* 2018;**4**:292−319.
392. Bhattacharya A, Seshadri M, Oven SD, Tóth K, Vaughan MM, Rustum YM. Tumor vascular maturation and improved drug delivery induced by methylselenocysteine leads to therapeutic synergy with anticancer drugs. *Clin Cancer Res* 2008;**14**:3926−32.
393. Taurin S, Nehoff H, Greish K. Anticancer nanomedicine and tumor vascular permeability; where is the missing link?. *J Control Release* 2012;**164**:265−75.
394. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, et al. Analysis of nanoparticle delivery to tumours. *Nat Rev Mater* 2016;**1**:1−12.
395. Ushijima C, Tsukamoto S, Yamazaki K, Yoshino I, Sugio K, Sugimachi K. High vascularity in the peripheral region of non-small cell lung cancer tissue is associated with tumor progression. *Lung Cancer* 2001;**34**:233−41.
396. Tanaka Y, Hongo K, Tada T, Sakai K, Kakizawa Y, Kobayashi S. Growth pattern and rate in residual nonfunctioning pituitary adenomas: correlations among tumor volume doubling time, patient age, and MIB-1 index. *J Neurosurg* 2003;**98**:359−65.

397. Rohner E, Yang R, Foo KS, Goedel A, Chien KR. Unlocking the promise of mRNA therapeutics. *Nat Biotechnol* 2022;**40**:1586−600.
398. Kong Q, Lin CG. Oxidative damage to RNA: mechanisms, consequences, and diseases. *Cell Mol Life Sci* 2010;**67**:1817−29.
399. Swayze EE, Siwkowski AM, Wancewicz EV, Migawa MT, Wyrzykiewicz TK, Hung G, et al. Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. *Nucleic Acids Res* 2007;**35**:687−700.
400. De A, Ko YT. Why mRNA-ionizable LNPs formulations are so short-lived: causes and way-out. *Expert Opin Drug Deliv* 2023;**20**:175−87.
401. Ball RL, Bajaj P, Whitehead KA. Achieving long-term stability of lipid nanoparticles: examining the effect of pH, temperature, and lyophilization. *Int J Nanomed* 2017;**12**:305−15.
402. Zhang H, Zhang L, Lin A, Xu C, Li Z, Liu K, et al. Algorithm for optimized mRNA design improves stability and immunogenicity. *Nature* 2023;**621**:396−403.
403. Kumar K, Rani V, Mishra M, Chawla R. New paradigm in combination therapy of siRNA with chemotherapeutic drugs for effective cancer therapy. *Curr Res Pharmacol Drug Discov* 2022;**3**:100103.
404. Becquart C. *Quantitative NanoSIMS provides subcellular concentration and distribution of oligonucleotide therapeutics*, vol. 15; 2023.
405. Juliano RL. Chemical manipulation of the endosome trafficking machinery: implications for oligonucleotide delivery. *Biomedicines* 2021;**9**:512.
406. Edwards DK, Jasny E, Yoon H, Horscroft N, Schanen B, Geter T, et al. Adjuvant effects of a sequence-engineered mRNA vaccine: translational profiling demonstrates similar human and murine innate response. *J Transl Med* 2017;**15**:1.
407. Gao S, Dagnaes-Hansen F, Nielsen EJB, Wengel J, Besenbacher F, Howard KA, et al. The effect of chemical modification and nanoparticle formulation on stability and biodistribution of siRNA in mice. *Mol Ther* 2009;**17**:1225−33.
408. Lin Y, Guan X, Su J, Chen S, Fu X, Xu X, et al. Cell membrane-camouflaged nanoparticles mediated nucleic acids delivery. *Int J Nanomed* 2023;**18**:8001−21.
409. Chen BQ, Zhao Y, Zhang Y, Pan YJ, Xia HY, Kankala RK, et al. Immune-regulating camouflaged nanoplatforms: a promising strategy to improve cancer nano-immunotherapy. *Bioact Mater* 2022;**21**:1−19.
410. Seok H, Lee H, Jang ES, Chi SW. Evaluation and control of miRNA-like off-target repression for RNA interference. *Cell Mol Life Sci* 2018;**75**:797−814.
411. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, et al. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol* 2003;**21**:635−7.
412. Koller E, Propp S, Murray H, Lima W, Bhat B, Prakash TP, et al. Competition for RISC binding predicts *in vitro* potency of siRNA. *Nucleic Acids Res* 2006;**34**:4467−76.
413. Hannus M, Beitzinger M, Engelmann JC, Weickert MT, Spang R, Hannus S, et al. siPools: highly complex but accurately defined siRNA pools eliminate off-target effects. *Nucleic Acids Res* 2014;**42**:8049−61.
414. Sabourian P, Yazdani G, Ashraf SS, Frounchi M, Mashayekhan S, Kiani S, et al. Effect of physico-chemical properties of nanoparticles on their intracellular uptake. *Int J Mol Sci* 2020;**21**:8019.
415. Decuzzi P, Godin B, Tanaka T, Lee SY, Chiappini C, Liu X, et al. Size and shape effects in the biodistribution of intravascularly injected particles. *J Control Release* 2010;**141**:320−7.
416. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. *J Nanobiotechnol* 2022;**20**:262.
417. Wang Z, Wu Z, Liu J, Zhang W. Particle morphology: an important factor affecting drug delivery by nanocarriers into solid tumors. *Expert Opin Drug Deliv* 2018;**15**:379−95.
418. Li Y, Lian Y, Zhang LT, Aldousari SM, Hedia HS, Asiri SA, et al. Cell and nanoparticle transport in tumour microvasculature: the role of size, shape and surface functionality of nanoparticles. *Interf Focus* 2016;**6**:20150086.
419. Vedadghavami A, Zhang C, Bajpayee AG. Overcoming negatively charged tissue barriers: drug delivery using cationic peptides and proteins. *Nano Today* 2020;**34**:100898.
420. Yan Y, Liu XY, Lu A, Wang XY, Jiang LX, Wang JC. Non-viral vectors for RNA delivery. *J Control Release* 2022;**342**:241−79.
421. Jörgensen AM, Wibel R, Bernkop-Schnürch A. Biodegradable cationic and ionizable cationic lipids: a roadmap for safer pharmaceutical excipients. *Small* 2023;**19**:2206968.
422. Bose RJ, Lee SH, Park H. Lipid-based surface engineering of PLGA nanoparticles for drug and gene delivery applications. *Biomater Res* 2016;**20**:34.
423. Egbuna C, Parmar VK, Jeevanandam J, Ezzat SM, Patrick-Iwuanyanwu KC, Adetunji CO, et al. Toxicity of nanoparticles in biomedical application: nanotoxicology. *J Toxicol* 2021;**2021**:9954443.
424. Kisin ER, Murray AR, Keane MJ, Shi XC, Schwegler-Berry D, Gorelik O, et al. Single-walled carbon nanotubes: geno-and cytotoxic effects in lung fibroblast V79 cells. *J Toxicol Environ Health, Part A* 2007;**70**:2071−9.
425. Wang Y, Zhang B, Ding X, Du X. Dendritic mesoporous organosilica nanoparticles (DMONs): chemical composition, structural architecture, and promising applications. *Nano Today* 2021;**39**:101231.
426. Rahmati M, Mozafari M. Biological response to carbon-family nanomaterials: interactions at the nano-bio interface. *Front Bioeng Biotechnol* 2019;**7**:4.
427. Ding J, Zhou Y, Khan MS, Sy RN, Khosa F. Representation of sex, race, and ethnicity in pivotal clinical trials for dermatological drugs. *Int J Womens Dermatol* 2021;**7**:428−34.
428. Conde J, Arnold CE, Tian F, Artzi N. RNAi nanomaterials targeting immune cells as an anti-tumor therapy: the missing link in cancer treatment?. *Mater Today* 2016;**19**:29−43.
429. Shah P, Lalan M, Barve K. Intranasal delivery: an attractive route for the administration of nucleic acid based therapeutics for CNS disorders. *Front Pharmacol* 2022;**13**:974666.
430. Conte G, Costabile G, Baldassi D, Rondelli V, Bassi R, Colombo D, et al. Hybrid lipid/polymer nanoparticles to tackle the cystic fibrosis mucus barrier in siRNA delivery to the lungs: does PEGylation make the difference?. *ACS Appl Mater Inter* 2022;**14**:7565−78.
431. Nof E, Zidan H, Artzy-Schnirman A, Mouhadeb O, Beckerman M, Bhardwaj S, et al. Human multi-compartment airways-on-chip platform for emulating respiratory airborne transmission: from nose to pulmonary acini. *Front Physiol* 2022;**13**:853317.
432. Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, et al. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicol Sci* 2000;**55**:24−35.
433. Wang G, Chen S, Qiu N, Wu B, Zhu D, Zhou Z, et al. Virus-mimetic DNA-ejecting polyplexes for efficient intracellular cancer gene delivery. *Nano Today* 2021;**39**:101215.
434. Ramot Y, Rotkopf S, Gabai RM, Zorde Khvalevsky E, Muravnik S, Marzoli GA, et al. Preclinical safety evaluation in rats of a polymeric matrix containing an sirna drug used as a local and prolonged delivery system for pancreatic cancer therapy. *Toxicol Pathol* 2016;**44**:856−65.
435. Suda T, Liu D. Hydrodynamic gene delivery: its principles and applications. *Mol Ther* 2007;**15**:2063−9.
436. Dul M, Stefanidou M, Porta P, Serve J, O'Mahony C, Malissen B, et al. Hydrodynamic gene delivery in human skin using a hollow microneedle device. *J Control Release* 2017;**265**:120−31.
437. Waldmann TA. Cytokines in cancer immunotherapy. *Cold Spring Harb Perspect Biol* 2018;**10**:a028472.
438. Wang D, Wang T, Xu Z, Yu H, Feng B, Zhang J, et al. Cooperative treatment of metastatic breast cancer using host−guest nanoplatform coloaded with docetaxel and siRNA. *Small* 2016;**12**:488−98.
439. Makkouk A, Weiner G. Cancer Immunotherapy and breaking immune tolerance-new approaches to an old challenge. *Cancer Res* 2015;**75**:5−10.
440. Zhou F, Huang L, Li S, Yang W, Chen F, Cai Z, et al. From structural design to delivery: mRNA therapeutics for cancer immunotherapy. *Exploration* 2024;**4**:20210146.

441. De Keersmaecker B, Claerhout S, Carrasco J, Bar I, Corthals J, Wilgenhof S, et al. TriMix and tumor antigen mRNA electroporated dendritic cell vaccination plus ipilimumab: link between T-cell activation and clinical responses in advanced melanoma. *J Immunother Cancer* 2020;**8**:e000329.
442. Papachristofilou A, Hipp MM, Klinkhardt U, Früh M, Sebastian M, Weiss C, et al. Phase Ib evaluation of a self-adjuvanted protamine formulated mRNA-based active cancer immunotherapy, BI1361849 (CV9202), combined with local radiation treatment in patients with stage IV non-small cell lung cancer. *J Immunother Cancer* 2019;**7**:1−14.
443. Guo Y, Zhang Q, Zhu Q, Gao J, Zhu X, Yu H, et al. Copackaging photosensitizer and PD-L1 siRNA in a nucleic acid nanogel for synergistic cancer photoimmunotherapy. *Sci Adv* 2022;**8**:eabn2941.
444. Bialkowski L, van Weijnen A, Van der Jeught K, Renmans D, Daszkiewicz L, Heirman C, et al. Intralymphatic mRNA vaccine induces CD8 T-cell responses that inhibit the growth of mucosally located tumours. *Sci Rep* 2016;**6**:22509.
445. Patel MR, Bauer TM, Jimeno A, Wang D, LoRusso P, Do KT, et al. A phase I study of mRNA-2752, a lipid nanoparticle encapsulating mRNAs encoding human OX40L, IL-23, and IL-36γ, for intratumoral (iTu) injection alone and in combination with durvalumab. *J Clin Oncol* 2020;**38**:3092.
446. Lin L, Kane N, Kobayashi N, Kono EA, Yamashiro JM, Nickols NG, et al. High-dose per fraction radiotherapy induces both antitumor immunity and immunosuppressive responses in prostate tumors. *Clin Cancer Res* 2021;**27**:1505−15.
447. Shibata SI, Doroshow JH, Frankel P, Synold TW, Yen Y, Gandara DR, et al. Phase I trial of GTI-2040, oxaliplatin, and capecitabine in the treatment of advanced metastatic solid tumors: a California cancer consortium study. *Cancer Chemother Pharmacol* 2009;**64**:1149−55.
448. Peeters M, Verbruggen L, Teuwen L, Vanhoutte G, Vande Kerckhove S, Peeters B, et al. Reduced humoral immune response after BNT162b2 coronavirus disease 2019 messenger RNA vaccination in cancer patients under antineoplastic treatment. *ESMO Open* 2021;**6**:100274.
449. Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: an update. *J Gene Med* 2018;**20**:e3015.
450. Bezeljak U. Cancer gene therapy goes viral: viral vector platforms come of age. *Radiol Oncol* 2022;**56**:1−13.
451. US Food and Drug Administration. Chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs). *Guidance for industry* 2008. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug. [Accessed 15 June 2024].
452. Byrnes AP, Huang Y, Shannon K. The FDA review process for cancer gene therapy. In: Lattime EC, Gerson SL, editors. *Gene therapy of cancer.* 3rd ed. Amsterdam: Elsevier; 2013. p. 503−15.
453. Bailey AM, Arcidiacono J, Benton KA, Taraporewala Z, Winitsky S. United States Food and Drug Administration regulation of gene and cell therapies. In: Galli MC, Serabian M, editors. *Regulatory aspects of gene therapy and cell therapy products: a global perspective*. Switzerland: Springer; 2015. p. 1−29. Cham.
454. Humphreys SC, Davis JA, Iqbal S, Kamel A, Kulmatycki K, Lao Y, et al. Considerations and recommendations for assessment of plasma protein binding and drug−drug interactions for siRNA therapeutics. *Nucleic Acids Res* 2022;**50**:6020−37.
455. Rolls MM, Webster P, Balba NH, Rose JK. Novel infectious particles generated by expression of the vesicular stomatitis virus glycoprotein from a self-replicating RNA. *Cell* 1994;**79**:497−506.
456. Cheng Y, Wei H, Tan JY, Peeler DJ, Maris DO, Sellers DL, et al. Nano-sized sunflower polycations as effective gene transfer vehicles. *Small* 2016;**12**:2750−8.
457. Hosseinpour S, Cao Y, Liu J, Xu C, Walsh LJ. Efficient transfection and long-term stability of rno-miRNA-26a-5p for osteogenic differentiation by large pore sized mesoporous silica nanoparticles. *J Mater Chem B* 2021;**9**:2275−84.
458. Stitz L, Vogel A, Schnee M, Voss D, Rauch S, Mutzke T, et al. A thermostable messenger RNA based vaccine against rabies. *Plos Negl Trop Dis* 2017;**11**:e0006108.
459. Esteban-Fernández de Ávila B, Angell C, Soto F, Lopez-Ramirez MA, Báez DF, Xie S, et al. Acoustically propelled nanomotors for intracellular siRNA delivery. *Acs Nano* 2016;**10**:4997−5005.
460. Simó C, Serra-Casablancas M, Hortelao AC, Di Carlo V, Guallar-Garrido S, Plaza-García S, et al. Urease-powered nanobots for radionuclide bladder cancer therapy. *Nat Nanotechnol* 2024;**19**:554−64.