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REVIEW

Lipid Nanovesicles in Cancer Treatment: Improving Targeting and Stability of Antisense Oligonucleotides

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Abstract: Cancer remains a leading cause of mortality worldwide, accounting for approximately 10 million deaths annually. Standard treatments, including surgery, radiotherapy, and chemotherapy, often result in damage to healthy cells and severe toxic side effects. In recent years, antisense technology therapeutics, which interfere with RNA translation through complementary base pairing, have emerged as promising approaches for cancer treatment. Despite the availability of various antisense oligonucleotide (ASO) drugs on the market, challenges such as poor active targeting and susceptibility to clearance by circulating enzymes remain. Compared with other delivery systems, lipid nanovesicle (LNV) delivery systems offer a potential solution that uniquely enhances ASO targeting and stability. Studies have shown that LNVs can increase the accumulation of ASOs in tumor sites several-fold, significantly reducing systemic toxic reactions and demonstrating increased therapeutic efficiency in preclinical models. Additionally, LNVs can protect ASOs from enzymatic degradation within the body, extending their half-life and thus enhancing their therapeutic effects. This paper provides a comprehensive review of recent examples and applications of LNV delivery of ASOs in cancer treatment, highlighting their unique functions and outcomes. Furthermore, this paper discusses the key challenges and potential impacts of this innovative approach to cancer therapy.

Keywords: cancer therapy, antisense oligonucleotides, lipid nanovesicles, liposomes, extracellular vesicles, cell membrane vesicles

Introduction

Despite significant advances in science and technology, cancer remains a leading cause of mortality, accounting for a substantial proportion of human deaths.[1] Consequently, cancer treatment is a critical focus in clinical research. Currently, the mainstays of cancer treatment are surgery, chemotherapy, and radiotherapy. However, the metastasis, recurrence, and heterogeneity of cancer cells, as well as their resistance to chemotherapy and radiotherapy, have rendered conventional treatments ineffective for many types of cancers.[2]

In recent years, RNA-based therapeutics have demonstrated promising outcomes in clinical trials. By targeting non-druggable genes as therapeutic targets, these therapies illustrate the immense potential of RNA-based approaches in cancer treatment and offer considerable promise for future applications.[3]

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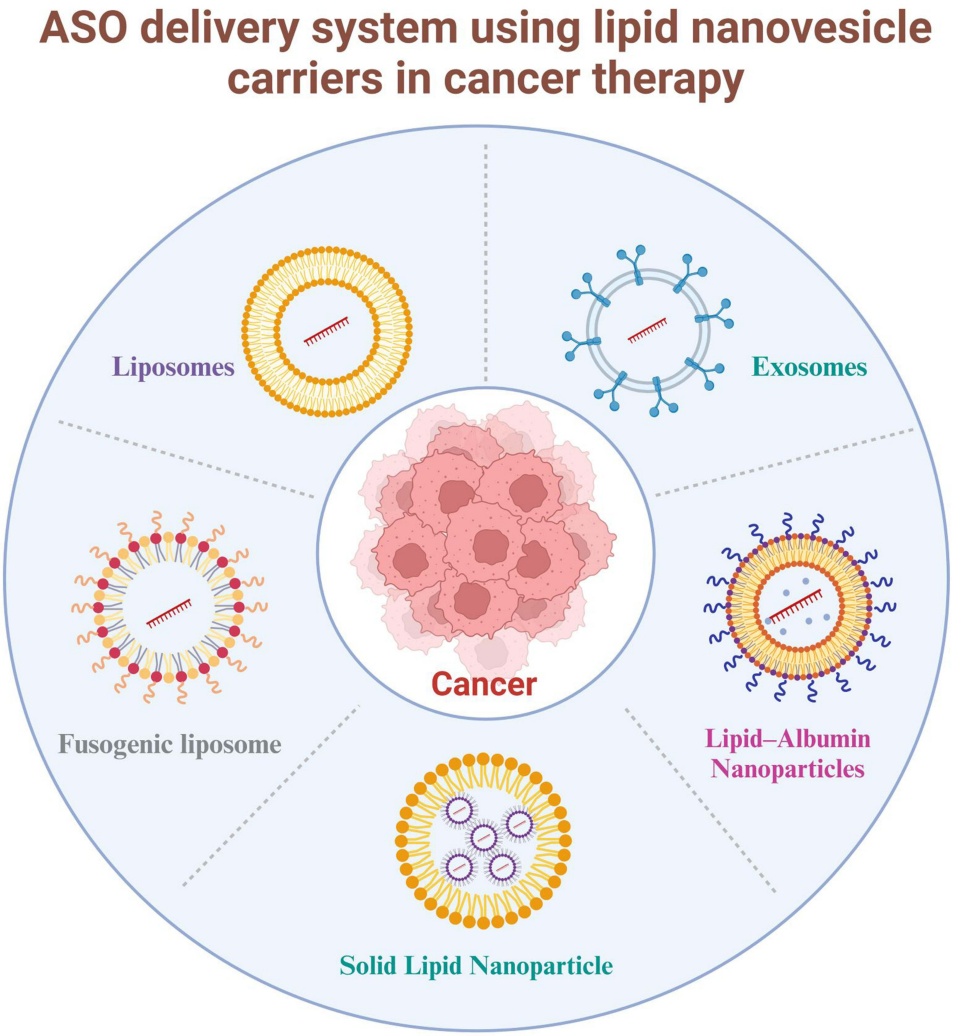
Graphical Abstract



RNA therapeutics utilize messenger RNA (mRNA) and noncoding RNA (ncRNA) as templates to synthesize nucleotide molecules that can form complementary base pairs, thereby regulating transcriptional and post-transcriptional processes. The primary nucleotide molecules in question are largely antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and microRNAs (miRNAs).[4] Among these, ASOs are single-stranded DNA or RNA molecules comprising 15–21 nucleotides that bind to RNA or protein targets to regulate protein expression or function. The field of ASO-based therapeutics is rapidly evolving, with ASOs playing a key role in treating diseases such as cancer,[5,6] Alzheimer's disease,[7] and diabetes.[8] To date, the FDA has approved twelve ASO drugs, and over one hundred ASO therapies have entered clinical trials, collectively indicating highly promising applications.

Despite the great potential of ASOs for drug development targeting non-pharmacological gene targets, several challenges remain. These include intra- and extracellular barriers and RNase degradation, which limit the efficacy of ASO therapies in vivo, particularly in systemic drug delivery. Various drug delivery systems, such as polymer nanoparticles, dendritic polymers, metal complexes, and lipid nanoparticles, have been developed to overcome these

barriers. However, polymer nanoparticles, dendritic polymers, and metal complexes still face problems such as biocompatibility and potential toxicity.

In contrast, lipid nanocapsules offer unique advantages in overcoming these challenges. By constructing nanodelivery systems, the stability and targeting of ASOs can be enhanced, and their immunogenicity can be reduced. Studies have shown that lipid nanocarriers can increase ASO accumulation at the tumor sites several-fold, significantly reduce the systemic toxicity response, and demonstrate increased therapeutic efficiency in preclinical models. In addition, LNPs can protect ASOs from enzymatic degradation in vivo and prolong their half-life, thus increasing their therapeutic efficacy.

This review underscores contemporary advancements in the realm of lipid nanocarrier delivery of ASOs for cancer therapy, offering a comprehensive synopsis of the present state of the technology; it meticulously delineates the current status and emergent trends in developing lipid nanocapsule delivery systems associated with ASOs in the domain of oncology therapy. Furthermore, it methodically analyzes the opportunities and challenges posed by this approach in the context of translational oncology therapy.

Mechanism of ASOs

Previous studies have demonstrated that ASOs of 16–20 nucleotides in length can target complementary RNAs through Watson–Crick base pairing, with minimal impact on the behavior of the target RNAs.[9] Upon binding to a specific RNA, oligonucleotides modulate RNA function through various mechanisms. The current mechanisms of action for ASOs can be categorized into two main groups: those promoting RNA cleavage and degradation and those solely occupying space through steric hindrance.

RNA Degradation Mechanisms

The RNase-H1 enzyme is an endogenous nuclease capable of specifically cleaving RNA–DNA-like double strands and subsequently releasing intact DNA. ASOs are designed to mimic this RNA–DNA pairing to facilitate RNase–H-mediated cleavage of RNA transcripts while releasing the intact ASOs to bind new transcripts.[10] The ASO mechanism can target mRNAs in both the cytoplasm and nucleus, in contrast to siRNAs, which are limited to acting on mRNAs in the cytoplasm.[11] Currently, RNase H1-mediated cleavage and degradation are widely used in FDA-approved ASO drugs that contain 8–10 consecutive deoxyribonucleotides with 2’ modifications. The rationale for this structural design is that the RNA-ASO complex must have at least 5 consecutive deoxyribonucleotide residues to serve as the substrate for the RNase-H1 enzyme. Enzyme activity is optimized when the RNA-ASO complex has 8–10 consecutive deoxyribonucleotides.[11] Furthermore, the 2’-position modification serves to protect the molecule from nuclease degradation while concomitantly increasing its activity[12] (Figure 1A).

Occupancy-Only Mechanisms

Occupancy-only mechanisms can both upregulate and downregulate gene expression, and there are several ways to achieve this. Among these mechanisms, ASOs enter the nucleus of target cells and bind to pre-mRNAs, splicing exons through a spatial site-blocking effect via different splicing methods, thus generating multiple different mRNAs and corresponding proteins.[13,14] The spliceosome’s splice position can be modified to selectively exclude (exon skipping, splice-out) or retain (exon inclusion, splice-in) specific exons.[15–17] An alternative approach is to utilize an occupancy-only mechanism to activate protein expression by interfering with the upstream open reading frames that negatively regulate translation;[18] this can also block the entry of mRNAs into the ribosome for protein translation, resulting in the downregulation of the expression of the genetic information carried by such mRNAs[12] and ultimately leading to the treatment of the disease (Figure 1B, D and E).

Antisense miRNA (Anti-miRNA)

MiRNAs are posttranscriptional gene regulators of 19–24 nucleotides in length that cause translational repression or mRNA deadenylation through complementary binding to the 3′ untranslated region (3′ UTR) of mRNA.[19] ASOs can be used to target miRNAs, which can then increase or inhibit translation, thereby regulating protein expression. These oligonucleotides are also known as anti-miRNA oligonucleotides (AMOs).[20] It has been demonstrated that AMOs bind to

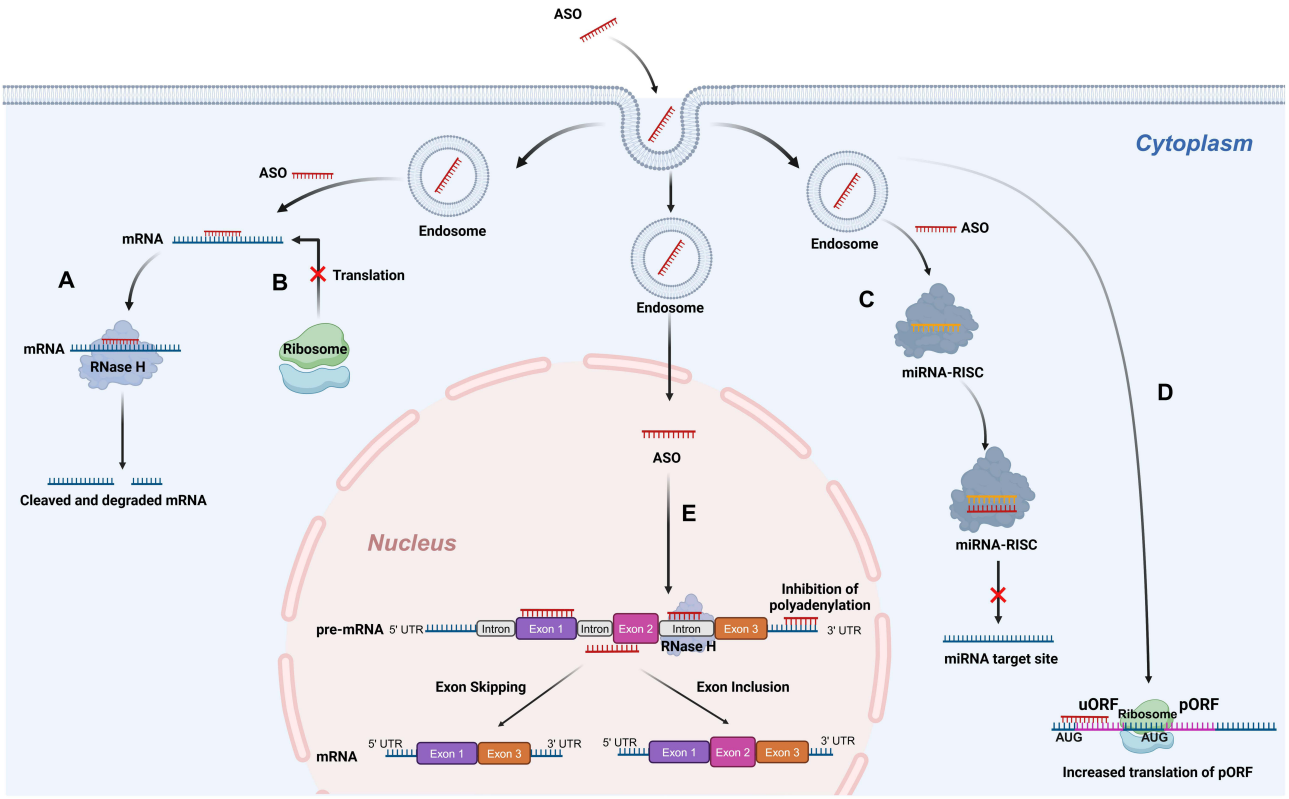


Figure 1 Mechanism of action of ASOs. When ASOs enter the cytoplasm, (A) they can degrade the target mRNA through the RNase-H enzyme and (B) can block the binding site of the ribosome to the target mRNA, thereby inhibiting translation. (C) ASOs can also bind to miRNA to form miRNA-RISC complexes that cannot bind to the corresponding mRNA, thereby affecting downstream expression. (D) ASOs can also target the upstream open reading frame (uORF) to activate the translation of the primary open reading frame (pORF). After entering the nucleus (E) ASOs also downregulate or destroy mRNA by modifying polyadenylation or splicing of immature mRNA (pre mRNA). Created in BioRender. Huiyan, D. (2025) https://BioRender.com/s37i893.

the target miRNA of the RNA-induced silencing complex (RISC) within the cytoplasmic mRNA processing body. The subsequent inability of this RISC complex to bind to the binding site on the mRNA results in regulated mRNA cleavage, translational repression, or deadenylation.[21,22] AMOs are currently employed in the treatment of cardiovascular, atherosclerotic, diabetic, and cancerous diseases.[23–25] However, AMOs can elicit adverse immune responses through non-specific binding to toll-like receptors.[26,27] Consequently, developing nanodelivery systems with high stability, low off-target effects, and minimal immunogenicity is urgently needed to address this issue (Figure 1C).

ASOs and Potential Targets for Cancer Therapy

Since the initial approval of the first ASO-based drug, Fomivirsen (withdrawn due to safety concerns), in 1998, the FDA has approved a total of twelve ASOs (Table 1). However, the majority of approved ASOs are designed to treat diseases such as Duchenne muscular dystrophy and cytomegalovirus retinitis. To date, there are no FDA-approved ASO drugs for treating cancer. This is due to the potential of ASOs, which has driven researchers to explore the field of oncology. Oligonucleotide therapy has been investigated as a potential cancer treatment for decades, with promising results observed in vitro.[28] Many ASO drugs have already been tested in clinical trials. In fact, 58 ASO drugs for cancer treatment have entered clinical trials when queried by *ClinicalTrials.gov* using the search terms "ASO" and "tumor" (Table 2). However, the majority of these ASO therapies are currently focused on clinical phases I and II. Only seven have entered clinical phases II/III or III. This indicates that while the technology has potential, it also faces significant challenges. Based on the results of previous clinical trials, the limitations of ASOs have been identified and attributed to the following areas:

Table 1 FDA-Approved Oligonucleotide Therapeutics

Name	Market Name	Indication	Target	Modality	MOA	Year Approved
Fomivirsen	Vitravene	CMV retinitis	Cytomegalovirus IE2 mRNA	PS	Translation block	1998
Mipomersen	Kynamro	HoFH	ApoB mRNA	PS 2'-O-MOE, 5mC	RNase H degradation	2013
Defibrotide	Defitelio	VOD	Multiple mechanisms of action	Mixture of PO ssDNA and dsDNA	Sequence-independent mechanism of action	2016
Eteplirsen	Exondys 51	DMD	Exon 51 dystrophin pre-mRNA	PMO	Splicing modulation	2016
Nusinersen	Spinraza	SMA	SMN-2 mRNA	PS 2'-O-MOE, 5mC	Splicing modulation	2016
Inotersen	Tegsedi	hATTR	TTR mRNA	PS 2'-O-MOE	RNase H degradation	2018
Volanesorsen	Waylivra	FCS	ApoC-III mRNA	PS 2'-O-MOE	RNase H degradation	2019
Golodirsen	Vyondys 53	DMD	Exon 53 dystrophin pre-mRNA	PMO	Splicing modulation	2019
Viltolarsen	Viltepso	DMD	Exon 53 dystrophin pre-mRNA	PMO	Splicing modulation	2020
Casimersen	Amondys 45	DMD	Exon 45 dystrophin pre-mRNA	PMO	Splicing modulation	2021
Tofersen	Qalsody	ALS	Superoxide dismutase 1 mRNA	PS 2'-O-MOE	RNase H degradation	2023
Eplontersen	Wainue	ATTRv-PN	TTR mRNA	PS 2'-O-MOE	RNase H degradation	2023

Abbreviations: MOA, Mechanism of action, CMV, Cytomegalovirus, HoFH, Homozygous familial hypercholesterolemia, VOD, Veno-occlusive disease, DMD, Duchenne Muscular Dystrophy, SMA, Spinal Muscular Atrophy, hATTR, Hereditary transthyretin amyloidosis, FCS, Familial chylomicronemia syndrome, ALS, Amyotrophic lateral sclerosis, ATTRv-PN, ATTRv amyloidosis with polyneuropathy, ApoB, apolipoprotein B, SMN-2, Survival motor neuron-2, TTR, Transthyretin, ApoC-III, Apolipoprotein C-III, PS, Phosphorothioate, 2′-MOE, 2′-O-methoxyethyl, PO, Phosphodiester, ssDNA, single-stranded DNA, dsDNA, double-stranded DNA, PMO, Phosphorodiamidate morpholino oligonucleotide.

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Table 2 Antisense Oligonucleotide Drugs That Have Entered Clinical Trials for Cancer

Disease Category	Trials/Refs	Target	Phase	Brief Title
Solid Tumor	NCT00558545	XIAP	I/II	A Phase 1–2, XIAP Antisense AEG35156 With Weekly Paclitaxel in Patients With Advanced Breast Cancer
	NCT00557596		I/II	A Phase 1–2, XIAP Antisense AEG35156 With Gemcitabine in Patients With Advanced Pancreatic Cancer
	NCT03101839	KRAS	I	Phase I Dose-Escalation Study of AZD4785 in Patients With Advanced Solid Tumours
	NCT03300505	AR	I	ARRx in Combination With Enzalutamide in Metastatic Castration Resistant Prostate Cancer
	NCT02144051		I	Phase I Open Label Dose Escalation Study to Investigate the Safety & Pharmacokinetics of AZD5312 in Patients With Androgen Receptor Tumors
	NCT04504669	FOXP3	I	First Time in Human Study of AZD8701 With or Without Durvalumab in Participants With Advanced Solid Tumours
	NCT02417753	STAT3	II	AZD9150, a STAT3 Antisense Oligonucleotide, in People With Malignant Ascites
	NCT01839604		I	A Phase I/Ib Study of AZD9150 (ISIS-STAT3Rx) in Patients With Advanced/Metastatic Hepatocellular Carcinoma
	NCT04196257	L-Grb-2	I	BP1001-A in Patients With Advanced or Recurrent Solid Tumors
	NCT01120288	HIF-1	I	A Pilot Study of EZN-2968, an Antisense Oligonucleotide Inhibitor of HIF-1alpha, in Adults With Advanced Solid Tumors With Liver Metastases
	NCT00056173	RNR	I/II	Combination of Capecitabine and GTI-2040 in the Treatment of Renal Cell Carcinoma
	NCT04485949	IGF-1R	II	A Phase 2b Clinical Study With a Combination Immunotherapy in Newly Diagnosed Patients With Glioblastoma
	NCT00003236	C-raf, Pkc-α	II	Chemotherapy in Treating Women With Previously Treated Metastatic Breast Cancer
	NCT00024661	RAF-1	I	Study to Determine the Maximum Tolerated Dose of LErafAON in Patients With Advanced Solid Tumors
	NCT00005032	Bcl-2	I/II	Bcl-2 Antisense Oligodeoxynucleotide G3139 and Paclitaxel in Treating Patients With Recurrent Small Cell Lung Cancer
	NCT00085228		II	Docetaxel With or Without Oblimersen in Treating Patients With Hormone-Refractory Adenocarcinoma (Cancer) of the Prostate
	NCT00030641		II/III	Docetaxel With or Without Oblimersen in Treating Patients With Non-Small Cell Lung Cancer
	NCT00004870		II	Olimersen and Irinotecan in Treating Patients With Metastatic or Recurrent Colorectal Cancer
	NCT00017251		I	Combination Chemotherapy Plus Oblimersen in Treating Patients With Previously Untreated Extensive-Stage Small Cell Lung Cancer
	NCT00063934		I/II	Oblimersen Plus Doxorubicin and Docetaxel in Treating Patients With Metastatic or Locally Advanced Breast Cancer
	NCT00059813		II	Oblimersen and Interferon Alfa in Treating Patients With Metastatic Renal Cell Cancer
	NCT00016263		III	Dacarbazine With or Without Oblimersen (G3139) in Treating Patients With Advanced Malignant Melanoma
	NCT00543205		II/III	Pharmacokinetics of G3139 in Subjects With Advanced Melanoma, Including Those With Normal Hepatic Function and Those With Moderate Hepatic Impairment
	NCT00070343			Oblimersen and Dacarbazine in Treating Patients With Advanced Malignant Melanoma That Has Responded to Treatment on Clinical Trial GENTA-GM301
	NCT00543231		I	A Phase I Study of G3139 Subcutaneous in Solid Tumors
	NCT00636545		I	Genasense as a 2-hour Intravenous Infusion in Subjects With Solid Tumors
	NCT00054548		I	Combination Chemotherapy Plus Oblimersen in Treating Patients With Advanced Solid Tumors

(*Continued*)

Table 2 (Continued).

Disease Category	Trials/Refs	Target	Phase	Brief Title
	NCT01083615	Clusterin	III	A Study Evaluating the Pain Palliation Benefit of Adding Custirsen to Docetaxel Retreatment or Cabazitaxel as Second Line Therapy in Men With Metastatic Castrate Resistant Prostate Cancer (mCRPC)
	NCT00258375		II	OGX-011 and Docetaxel in Treating Women With Locally Advanced or Metastatic Breast Cancer
	NCT00054106		I	Hormone Therapy and OGX-011 Before Radical Prostatectomy in Treating Patients With Prostate Cancer
	NCT00471432		I	OGX-011 and Docetaxel in Treating Patients With Metastatic or Locally Recurrent Solid Tumors
	NCT02423590	HSP27	II	Study of Gemcitabine/Carboplatin First-line Chemotherapy ± Apatorsen in Advanced Squamous Cell Lung Cancers
	NCT00959868		I	A Study for Treatment of Superficial Bladder Cancer Using OGX-427
	NCT01120470		II	OGX-427 in Castration Resistant Prostate Cancer Patients
	NCT00487786		I	Safety Study of an Antisense Product in Prostate, Ovarian, NSCL, Breast or Bladder Cancer
	NCT01780545		II	Phase 2 Study of Docetaxel ± OGX-427 in Patients With Relapsed or Refractory Metastatic Bladder Cancer
	NCT06079346	TGF-β2	II/III	A Study of OT-101 With mFOLFIRINOX in Patients With Advanced and Unresectable or Metastatic Pancreatic Cancer
	NCT04862767		I	TASO-001 in Combination With Recombinant Interleukin-2(Aldesleukin) in Advanced or Metastatic Solid Tumor
	NCT00668499	VEGF	I/II	A Study of VEGF-Antisense Oligonucleotide in Combination With Pemetrexed and Cisplatin for the Treatment of Advanced Malignant Mesothelioma
	NCT05267899	Akt-1	I	A Phase I First in Human Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of WGI-0301 in Patients With Advanced Solid Tumors
Hematologic Tumors	NCT02549651	STAT3	I	MEDI4736 Alone and in Combination With Tremelimumab or AZD9150 in Adult Subjects With Relapsed/Refractory DLBCL (D4190C00023)
	NCT01563302		II	Phase 1/2, Open-label, Dose-escalation Study of IONIS-STAT3Rx, Administered to Patients With Advanced Cancers
	NCT01159028	L-Grb-2	I	Clinical Trial of BP1001 (L-Grb-2 Antisense Oligonucleotide) in CML, AML, ALL & MDS
	NCT02923986		I/II	Clinical Trial of BP1001 (Liposomal Grb2 Antisense Oligonucleotide) in Combination With Dasatinib in Patients With Ph + CML Who Have Failed TKI, Ph+ AML, Ph+ MDS
	NCT02781883		II	Clinical Trial of BP1001 in Combination With With Venetoclax Plus Decitabine in AML
	NCT04072458	Bcl-2	I	A Clinical Trial of BP1002 in Patients With Advanced Lymphoid Malignancies
	NCT02243124	p53	I	A Study of Aezea® (Cenersen) in Transfusion Dependent Anemia Associated With Myelodysplastic Syndrome (MDS)
	NCT00002592	C-myb	II	Chemotherapy and Bone Marrow Transplantation in Treating Patients With Chronic Myelogenous Leukemia
	NCT00780052		I	Infusional C-myb ASODN in Advanced Hematologic Malignancies (UPCC 04701)
	NCT00466583	HIF-1	I	Phase 1 Study of EZN-2968 Weekly in Adult Patients With Advanced Solid Tumors or Lymphoma
	NCT00078234	Bcl-2	I/II	Genasense® (Oblimersen Sodium), Fludarabine, and Rituximab in Subjects With Chronic Lymphocytic Leukemia
	NCT00017602		III	Dexamethasone With or Without Oblimersen in Treating Patients With Relapsed or Refractory Multiple Myeloma
	NCT00021749		I/II	Phase I/II Study of Genasense in Patients With Chronic Lymphocytic Leukemia
	NCT00070083		I	Oblimersen, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Treating Patients With Stage II, Stage III, or Stage IV Diffuse Large B-Cell Lymphoma

(*Continued*)

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Table 2 (Continued).

Disease Category	Trials/Refs	Target	Phase	Brief Title
	NCT00024440		III	Fludarabine and Cyclophosphamide With or Without Oblimersen in Treating Patients With Relapsed or Refractory Chronic Lymphocytic Leukemia
	NCT00080847		II	S0349 Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone With or Without Oblimersen in Treating Patients With Advanced Diffuse Large B-Cell Non-Hodgkin's Lymphoma
Uncertain	NCT00100672	RAF-1	I	Study to Determine the Maximum Tolerated Dose of LErafAON in Patients With Advanced Cancer
	NCT00024648		I	Study to Determine Maximum Tolerated Dose of LErafAON Combined With Radiotherapy in Patients With Advanced Malignancies

1. Due to their strong negative charge, ASOs are less permeable, which limits their ability to be actively internalized into cells.[29]
2. ASOs are readily degraded by endogenous nucleases present in serum.[30]
3. ASOs lack the capacity to be actively targeted to specific tissues or cells.[31]
4. It is subject to uptake by reticuloendothelial system (RES) phagocytes, protein interactions, and renal excretion.[32]
5. Toxic effects have been observed.[33]

In conclusion, developing safe and effective delivery strategies is imperative to fully realize the therapeutic potential of ASO drugs in cancer treatment. Researchers are currently modifying these strategies to overcome the existing limitations. N-acetylgalactoside (GalNAc) couplings are frequently utilized for liver-targeted delivery in the clinic; however, effectively targeting delivery to tumor regions other than the liver remains challenging. We will subsequently discuss various delivery strategies.[34]

Delivery Strategies for ASO Drugs

Chemical Modifications

FDA-approved ASOs are chemically modified to protect them from degradation by nucleic acid endonucleases and exonucleases in vivo. These modifications increase their affinity for target RNA/DNA sequences and reduce their immunogenicity in the body.[35] The third generation of chemical modifications has been developed, with the first being phosphorothioate (PS) modification. This modification involves replacing an unbridged oxygen in the phosphate group of the ASOs with a sulfur moiety, forming a PS analog.[36] This modified ASO degrades the corresponding RNA fragment exclusively through the RNase H1 cleavage mechanism.[37] As modified PS can be toxic because it nonspecifically binds to proteins, further iterations were performed to address this issue. The second generation retained the PS backbone while introducing 2' sugar modifications, including 2'-O-methyl (2'-OMe), 2'-O-methoxy-ethyl (2'-MOE), and 2'-fluoro (2'-F) modifications. These moieties form antisense oligonucleotides that are less toxic than phosphorothioate ASOs and exhibit slightly increased affinity for their complementary RNAs. However, the efficiency of inducing RNase H1 cleavage of the target RNA remains a significant drawback of second-generation oligonucleotides. To address this issue, GapmeR-designed ASOs were developed to improve the degradation of RNase H1-recruited nucleotides and enhance the binding affinity and endosomal resistance of sugar-modified nucleotides. Based on the GapmeR's design concept, the third-generation ASOs outperform the previous two generations in terms of nuclease resistance, binding affinity, cell penetration, potency, and reduction in off-target effects.[38] The third generation of chemical modifications mainly includes nucleobase modifications, bridging nucleic acids, and alternative backbones. Among the nucleobase modifications, cytosine analogs are widely used to attenuate the immune stimulation caused by the activation of toll-like receptors by CpG dinucleotide-extended PS-ASOs,[39] in parallel with increasing their hydrophobicity and affinity for RNA targets.[40] Four of the currently FDA-approved and cleared ASO analogs have PMO backbones, suggesting superior performance in

terms of efficacy, low immunogenicity, and stability. Since this paper focuses on applying LNV technology in the field of ASOs, the details of chemical modifications are not discussed further.

Lipid-Based ASO Delivery Systems

Liposomes are colloidal spherical structures composed of amphiphilic lipid molecules that self-assemble in solution and feature hydrophilic, hydrophobic, and bridging structural domains.[41] Liposomes, an optimal choice for nanodrug delivery systems, have shown excellent performance in delivering nucleic acid and small-molecule drugs due to their good drug delivery capabilities and biocompatibility.[42–44] ASOs can be effectively embedded in the hydrophilic structural domains of liposomes, which protects them from nuclease degradation and simultaneously improves the efficiency of cellular uptake of ASOs.[45]

Anionic lipids, cationic lipids, and pH-sensitive liposomes have been reported as carriers for ASO delivery.[46] While anionic liposomes repel negatively charged ASOs, leading to low drug loading, cationic liposomes are often preferred.[47] The molecular structure of cationic liposomes resembles that of natural lipids, with cationic head groups replacing the amphipathic or anionic head groups. They consist of a hydrophobic portion with two alkyl chains or cholesterol moieties, a positively charged polar head group, and a linker connecting the polar group to the hydrophobic portion. This positive charge enables electrostatic interactions with negatively charged cell membranes, improving their transfection efficiency and endosomal escape.[48] Li et al synthesized a cationic liposome for treating acute myeloid leukemia (AML) that incorporates deoxycholate-polyethyleneimine coupling (DOC-PEI). This cationic agent enhances the delivery efficiency of oligonucleotides by promoting endosomal membrane disruption. By modifying the liposome surface with an anti-CD33 scFv (aCD33) as a targeting ligand for AML, this delivery system offers a potential therapeutic approach for AML.[49] Pan et al developed a DCP (cytosine-based/cationic lipids DNCA/CLD and DSPE-PEG) cationic liposome delivery system loaded with CT102, which targets the human insulin-like growth factor type 1 receptor (IGF1R) gene to efficiently inhibit the proliferation of hepatocellular carcinoma (HCC) cells. This promising liposome delivery system for cancer combination therapy involves adjusting the compositional ratio of DNCA/CLD/PEG/ASO to obtain the optimal ratio of liver-targeted drug formulation and ideal extrahepatic accumulation conditions[50] (Figure 2). Overexpression of

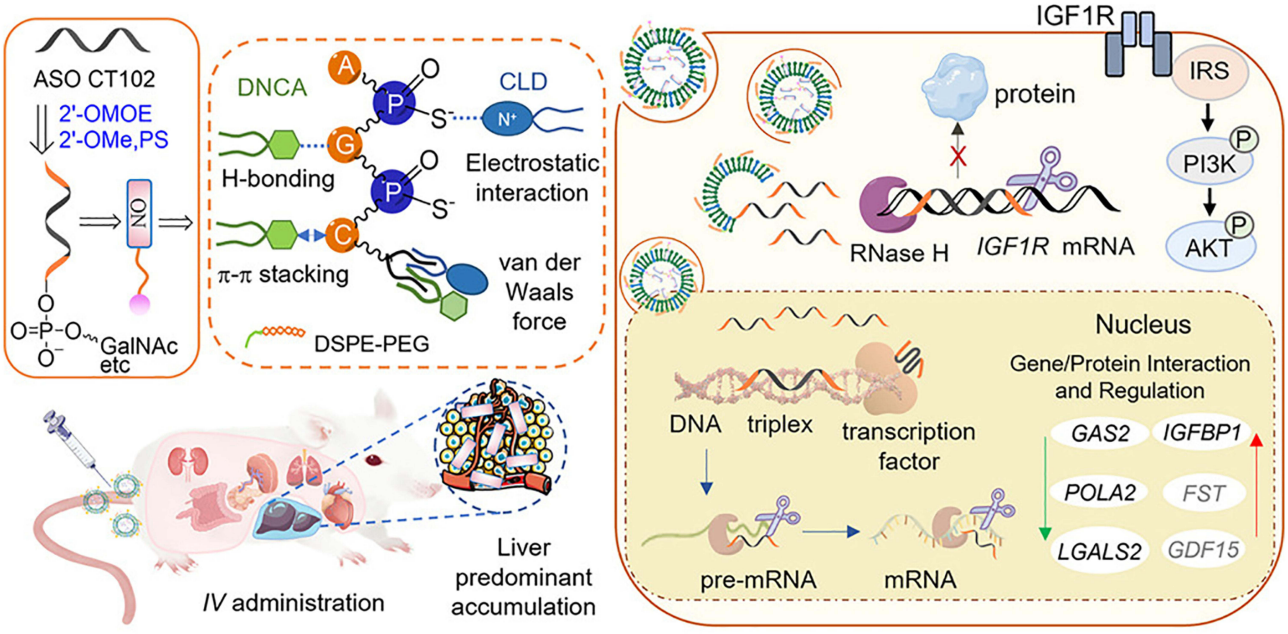

Figure 2 Modified ASO conjugates encapsulated with cytidinyl/cationic lipids exhibit more potent and longer-lasting anti-HCC effects. Yang et al constructed a series of gapmers/conjugates of an ASO CT102-targeting IGF1R mRNA. These ASOs could be delivered with DNCA/CLD/PEG lipids (DCP) predominantly to the liver, and showed more potent and longer-lasting anti-HCC efficacy by interacting or regulating more potential genes and proteins in the nucleus. Reprinted from Pan Y, Guan J, Gao Y, et al. Modified aso conjugates encapsulated with cytidinyl/cationic lipids exhibit more potent and longer-lasting anti-hcc effects. *Mol Ther Nucleic Acids*. 2023;32:807–821. Creative Commons.[50]

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translationally controlled tumor protein (TCTP) plays a critical role in castration-resistant prostate cancer (CRPCa), primarily through the interaction and negative feedback loop between TCTP and p53, which leads to the progression of prostate cancer (Pca).[51] Thus, silencing target mRNAs with ASOs prevents TCTP protein expression, potentially restoring the sensitivity of cancer cells to hormonal therapy and chemotherapy. However, ASOs lack tumor-specific targeting ability. Based on the expression of Her2 on the surface of PCa cells, the encapsulation of ASOs within anti-Her2 trastuzumab-modified cationic liposomes enables active targeting of PCa cells. The results showed that the lipid nanosystem could produce effective antiproliferative effects at longer exposure times.[52] Liposomes with drug-loaded liposomes covalently attached to monoclonal or genetically engineered antibodies on the surface are also referred to as immunoliposomes.[53]

Recently, ionizable liposomes have been synthesized in large quantities. Ionizable liposomes typically have a tertiary amine head and two carbon chain tails. The tertiary amine head acts to ionize lipids under acidic conditions, and the lipid carbon chain tails provide strong hydrophobicity to facilitate doping within the particles during nanoparticle formation.[54] Ionizable liposomes are only positively charged intracellularly because they typically have a pKa less than 7. These liposomes remain neutral under physiological conditions (pH≈7.4) and become protonated in acidic pH conditions (pH < 6.0). Lipid nanocarriers formed from ionizable lipids have an overall neutral surface charge, differentiating them from cationic lipids. This neutral charge reduces biotoxicity and circulation time, making ionizable liposomes a promising delivery system for ASOs in cancer treatment.[55]

Furthermore, given that the tumor region is hypoxic, the lactic acid secreted by anaerobic glycolysis in the (TME) results in a pH between 5.5 and 7.0.[56–58] This theoretical basis has prompted the development of pH-sensitive liposomes, designed to be stable at physiological pH but destabilize within the acidic TME, thereby promoting the release of their payload.[59] For example, Yao et al developed RA/RX pH-sensitive liposomes for colon cancer treatment, incorporating the cyclic peptide RA-v within the liposomal shell to induce apoptosis via the mitochondrial pathway.[60] In addition, ASOs in the core of RA/RX liposomes inhibit the expression of hypoxia-inducible factor 1α (HIF-1α) and couple death receptor 5-specific antibodies (anti-DR5) to the surface of liposomes for active targeting. These antibodies specifically recognize proapoptotic cell surface receptors on colorectal cancer cells. HIF-1α is associated with tumor angiogenesis, metastasis, proliferation, and metabolic reprogramming. Several studies have demonstrated that inhibiting HIF-1α expression enhances antitumor effects.[61,62] Since caspase-8 plays a pivotal role in apoptotic signaling pathways,[63,64] this delivery system enables therapeutic self-monitoring by dynamically visualizing the activation of caspase-8 in situ. This approach provides a novel, more efficacious strategy for combinatorial therapy of O_2-deficient tumors.[65]

Neutral liposomes, cationic liposomes, and lipid nanoparticles have advantages and limitations. Neutral liposomes are suitable for low-toxicity and high-stability applications, while cationic liposomes excel in nucleic acid delivery. Lipid nanoparticles provide a versatile and highly stable solution. Optimal delivery system selection depends on the specific therapeutic objectives and requirements.

Solid lipid nanoparticles (SLNs) represent an emerging submicron drug delivery system. They have a lipid core matrix that solubilizes lipophilic molecules and is stabilized by surfactants, controls the drug release rate, and improves cell membrane affinity.[66] Currently, SLNs are being explored to improve cancer therapy due to their ability to enhance drug delivery through blood vessels. [67,68] Shi et al synthesized an SLN loaded with anti-miR-21 ASO (AMO-CLOSs) for treating human lung cancer.[69] miR-21 is overexpressed in the A549 human lung cancer cell line, contributing to cell proliferation, apoptosis inhibition, and tumor metastasis.[70] AMO-CLOSs effectively deliver AMO to lung cancer cells, silencing overexpressed miR-21, inhibiting cell growth and apoptosis, and reducing cell invasion and migration.

In brief, lipid-based nanodelivery systems currently stand out as leading drug delivery systems due to their structural versatility, biocompatibility, biodegradability, nontoxicity, and nonimmunogenicity.[71] However, the stability of lipid-based nanodelivery systems for encapsulated drugs remains a key limiting factor for their drug delivery applications. In addition, large-scale production and development of effective sterilization techniques continue to be challenges that need to be addressed.[72] Overcoming these limitations may facilitate the achievement of superior therapeutic outcomes during ASO delivery.

Extracellular Vesicles Based ASO Delivery Systems

Extracellular vesicles (EVs) are lipid bilayer membrane vesicles secreted by living cells into the extracellular space at the nanometer scale.[73] They are categorized by particle size and include exosomes (30–150 nm), microvesicles (50–1000 nm), and apoptotic vesicles (100–5000 nm).[74] EVs present several distinctive characteristics, including inclusions from the source cell, which can influence the biological properties of the recipient cell. Compared with liposomes, EVs can be efficiently loaded with hydrophilic molecules, such as nucleic acids, to improve their loading efficiency. Additionally, the special proteins on the surface of EVs can prevent interactions with antibodies and coagulation factors in the blood, reducing immune responses in vivo.[75]

Furthermore, EV membranes, enriched in cholesterol, phosphatidylserine, sphingomyelin, and other sphingolipids, confer resistance to detergent solubilization and high temperatures.[76] Consequently, EVs exhibit greater stability in body fluids than liposomes.[77] Based on their inherent stability, immunotolerance, and nontoxicity, EVs are optimal for drug delivery in treating various diseases.[78–83]

Based on the tropism of human mesenchymal stem cells (MSCs) toward glioblastoma multiforme (GBM),[84] Jessian L Munoz et al demonstrated that MSC-secreted EVs delivered anti-miR-9 to GBM cells and significantly reduced MDR1 expression in TMZ-resistant GBM; this ultimately resulted in the reversal of GBM resistance, suggesting that EVs can serve as effective ASO carriers for cancer therapy.[85] MiR-142-3p plays a pivotal role in breast cancer proliferation, survival, and progression. Its inhibition downregulates the classical Wnt signaling pathway (also known as the Wnt/β-catenin signaling pathway) and decreases miR-150 expression in trans.[86] Zahra Naseri et al loaded anti-miRNA-142-3p into MSC-derived EVs, and their findings demonstrated that miRNA-142-3p and miRNA-150 were downregulated in EV-treated cells loaded with anti-miRNAs, resulting in enhanced therapeutic efficacy in breast cancer cells; this suggests that miRNA-142-3p downregulation is crucial for effective treatment.[87] miR-221 also plays a pivotal role in tumor proliferation and progression by inhibiting PTEN and cell cycle protein-dependent kinase inhibitor family members' expression.[88] Han et al loaded anti-miR-221 ASOs into neuropilin-1 (NRP-1)-targeted human umbilical cord mesenchymal cell-derived EVs, demonstrating the ability to actively target colorectal cancer cells. They also showed that the delivery system infiltrated anti-miR-221 AMO into solid tumors, significantly inhibiting the growth of colorectal cancer cells in vitro and in vivo[89] (Figure 3). In a recent study, Yu et al utilized human umbilical cord mesenchymal stem cell (hUC-MSC)-derived exosomes as carriers for the delivery of anti-miR-146b-5p ASO (PMO-146b), and CP05-PMO-146b was loaded onto the surface of EVs. ePPMO-146b successfully inhibited the progression of colorectal cancer by inhibiting the epithelial–mesenchymal transition in vitro and in vivo, suggesting that exosome-mediated delivery of ASOs has great potential for cancer therapy.[90]

Erythrocytes have been identified as a potential cellular source of EVs owing to their lack of nuclear and mitochondrial DNA, ease of accessibility, and abundance within the body (84% of all cells).[91] Waqas Muhammad Usman's team constructed an ASO-containing erythrocyte extracellular vesicle (RBCEV) for treating leukemia and breast cancer[92] (Figure 4). miR-125b has been demonstrated to promote the development of leukemia and breast cancer by inhibiting the p53 oncogenic network, particularly in acute myeloid leukemia and chemoresistant breast tumors.[93,94] This delivery system effectively treats leukemia cells in the liver and spleen, where leukemia commonly occurs, by intraperitoneal injection and antagonizing breast cancer cells by intratumoral injection without any observable side effects. Chen et al also used vesicle-loaded exogenous drugs and functionalized vesicle surfaces to treat AML.[95] CD33, a member of the sialic acid–binding immunoglobulin-like lectin family, is highly expressed on most AML cells. The uptake specificity of this vesicle by AML cells can be improved by modifying the surface of the nanovesicles with an anti-CD33 monoclonal antibody.[96] FMS-like tyrosine kinase 3 (FLT3) mutations are the most prevalent in AML patients, conferring an elevated risk of relapse and reducing overall and disease-free survival in AML patients.[97] When targeted for miR-125b and FLT3 mutations, anti-FLT3-ITD and anti-miR-125b ASOs were loaded into RBCEVs, and the results revealed synergistic antitumor effects of these two ASOs in combination treatment.

Tumor immunotherapy is a therapeutic method that enables the body to generate tumor-specific immune responses through active or passive means; it functions to suppress and kill tumor cells, which has become a new hotspot in cancer treatment due to its advantages of specificity, high efficiency, and freedom of the body from injurious treatments.[98] The

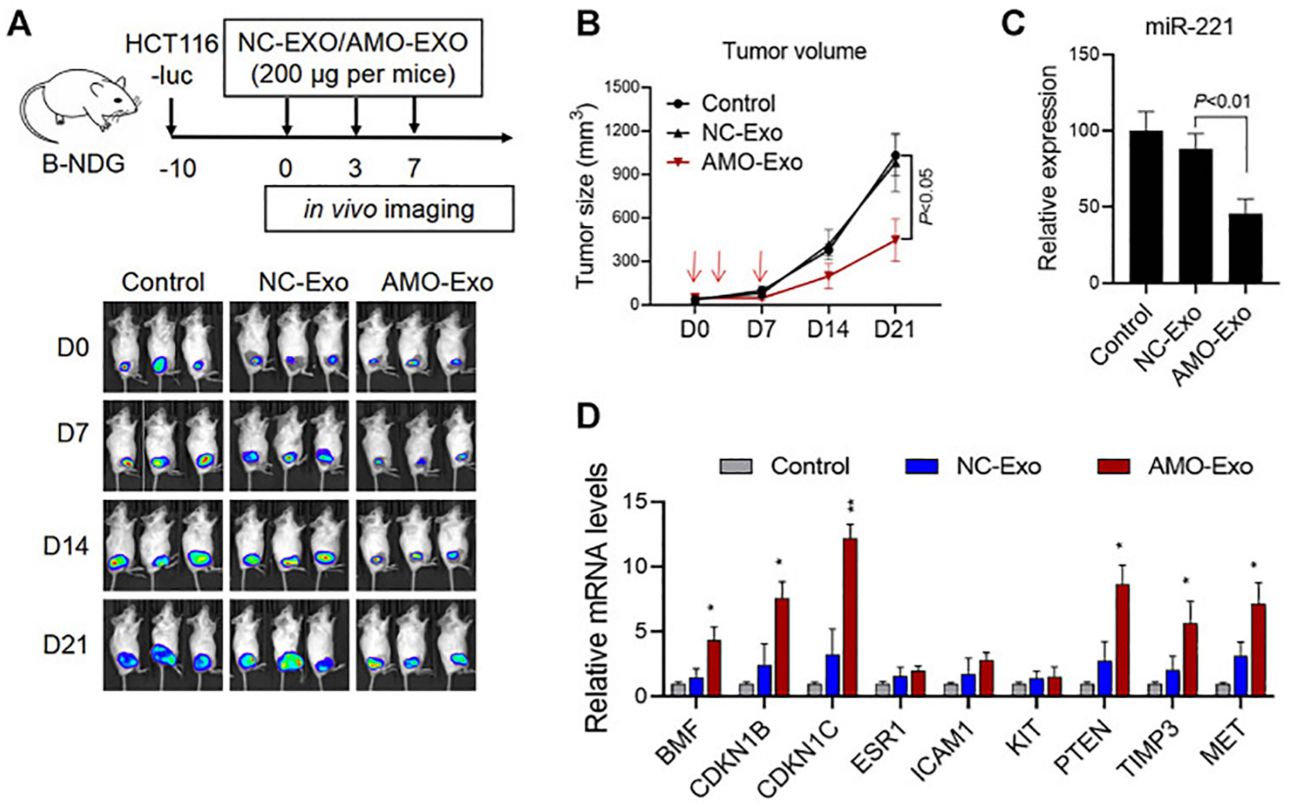

Figure 3 AMO-loaded exosomes suppressed tumor growth in vivo. (**A**) HCT116 tumor-bearing mice received 3 intratumoral injections of exosome drugs on days 0, 3, and 7. In vivo imaging of the mice was performed once a week to observe the change of fluorescence value. (**B**) Tumor changes in mice were measured with a vernier caliper. (**C**) The level of miR-221 in the dissected mouse tumor tissues was detected by quantitative PCR. (**D**) The mRNA levels of downstream target genes of miR-221 in the dissected mouse tumor tissues were detected by quantitative PCR. *$p < 0.05$, **$p < 0.01$. Reprinted from Han S, Li G, Jia M, et al. Delivery of anti-miRNA-221 for colorectal carcinoma therapy using modified cord blood mesenchymal stem cells-derived exosomes. *Front Mol Biosci*. 2021;8:743013. Creative Commons.[89]

field of immune checkpoint-related therapies has garnered significant interest in recent years. However, approximately 85% of patients fail to achieve a durable objective tumor response, partly due to tumor immune escape mechanisms.[99] Tumor-associated macrophages (TAMs) within the TME represent the primary cause of resistance to immune checkpoint inhibitor therapy.[100] TAMs are macrophages with an M2 phenotype that suppress the immune response and promote tumorigenesis and development.[101] ExoASO-STAT6 is an exosome delivery system prepared for treating colorectal and hepatocellular carcinoma by Sushrut Kamerkar et al (Figure 5). This system delivers ASOs targeting STAT6 to TAMs and STAT6 downregulation reprograms TAMs to the M1 phenotype, which significantly enhances anti-tumor immune responses.[102] Consequently, exoASO-STAT6 represents a promising new strategy for targeting myeloid cells in cancer.

In conclusion, as emerging nanocarriers, EVs can potentially reduce immunogenicity, enhance biocompatibility, and target delivery to specific cells and tissues. Clinical translation remains challenging despite their considerable research value as ASO delivery vehicles. Further studies are needed to explore the scalability, safety, and clinical validation of EV-based therapies. Since EVs are derived from cells, the processes of cell culture and extraction are complex and time-consuming, large-scale generation remains a great challenge, and the quality control of EVs is still not fully harmonized internationally. Developing appropriate international standards is crucial for ensuring the safety and efficacy of EV products. However, with improvements in EV production processes and equipment, as well as researchers' deeper understanding of tumor development, developing ASO-related products using EVs as carriers will continue to evolve. This progress is expected to revolutionize cancer treatments and advance precision medicine.

Cell Membrane-Based Delivery Systems

Cell membrane-derived LNVs are vesicles that form directly from the cell membrane after protrusion and separation. These vesicles contain only the cell's lipid and protein components and no cell-derived inclusions. Since these vesicles

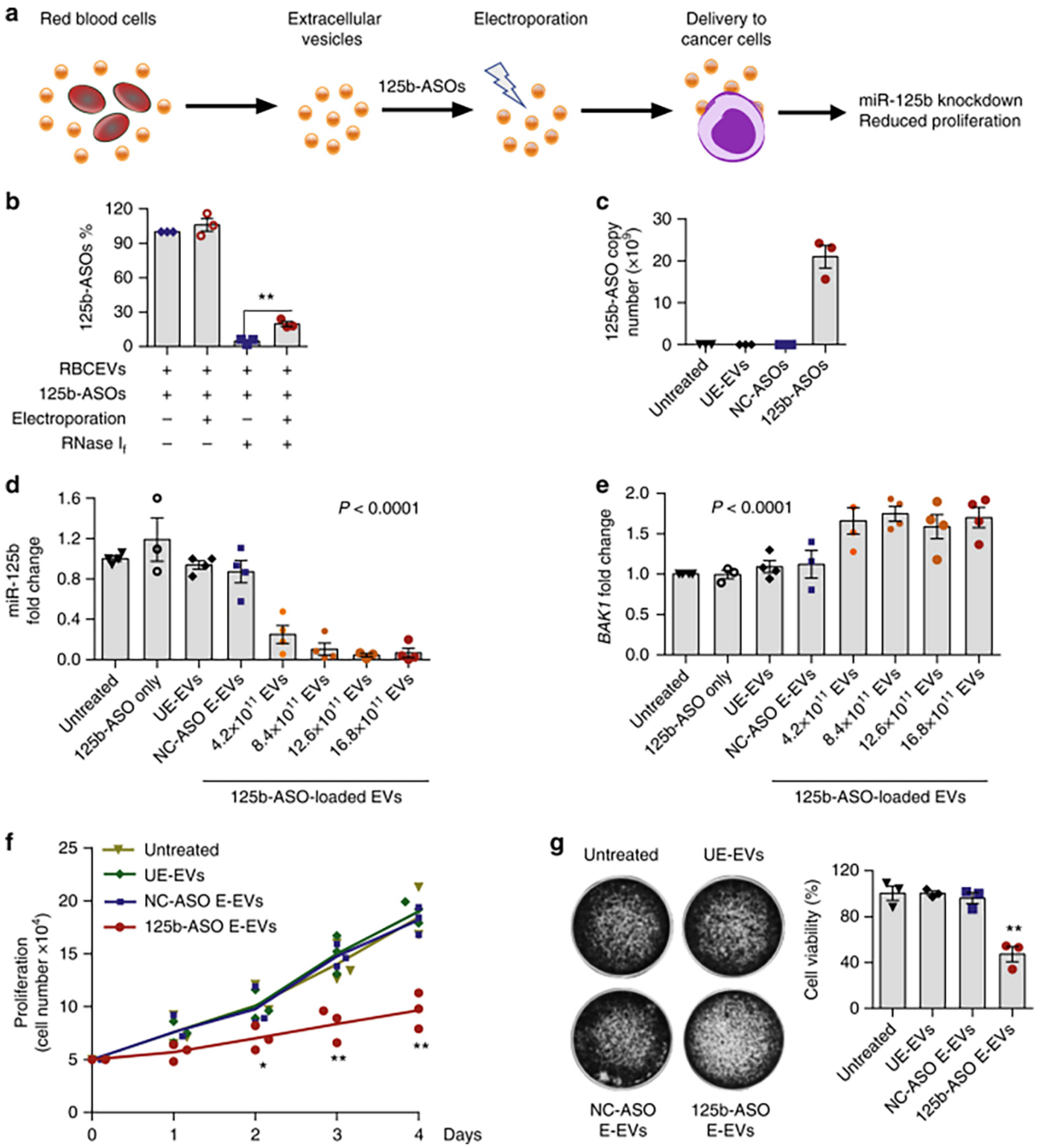

Figure 4 RBCEVs deliver ASOs to leukemia and breast cancer cells for miR-125b inhibition. (**a**) Experimental scheme of ASOs delivery to cancer cells using RBCEVs. (**b**) Percentage of anti-miR-125b ASOs (125b-ASOs) associated with 6.2×10^{11} unelectroporated or 125b-ASO-electroporated RBCEVs after a treatment with RNase If for 30 min. (**c**) Copy number of 125b-ASO in MOLM13 cells treated with 12.4×10^{11} RBCEVs unelectroporated (UE-EVs) or RBCEVs electroporated with NC-ASOs or with 125b-ASOs for 72 h. (**d**) Expression fold change of miR-125b in MOLM13 cells that were incubated with 125b-ASOs alone, 16.8×10^{11} unelectroporated RBCEVs (UE-EVs), 16.8×10^{11} NC-ASOs-loaded RBCEVs, or 4.2 to 16.8×10^{11} 125b-ASOs loaded RBCEVs. miR-125b expression was determined using TaqMan qRT-PCR normalized to U6b RNA and presented as average fold change relative to the untreated control. (**e**) Expression fold change of BAK1 in MOLM13 cells treated as in d, determined using SYBR Green qRT-PCR, normalized to GAPDH and presented as average fold change relative to the untreated control. (**f**) Proliferation of MOLM13 cells treated with 12.4×10^{11} unelectroporated or NC/125b-ASO-electroporated EVs, determined using cell counts. (**g**) Viability of breast cancer CA1a cells (%) treated as in f, determined by crystal violet staining. *$p < 0.05$, **$p < 0.01$. Reprinted from Usman WM, Pham TC, Kwok YY, et al. Efficient RNA drug delivery using red blood cell extracellular vesicles. *Nat Commun*. 2018;9(1):2359. Creative Commons.[92]

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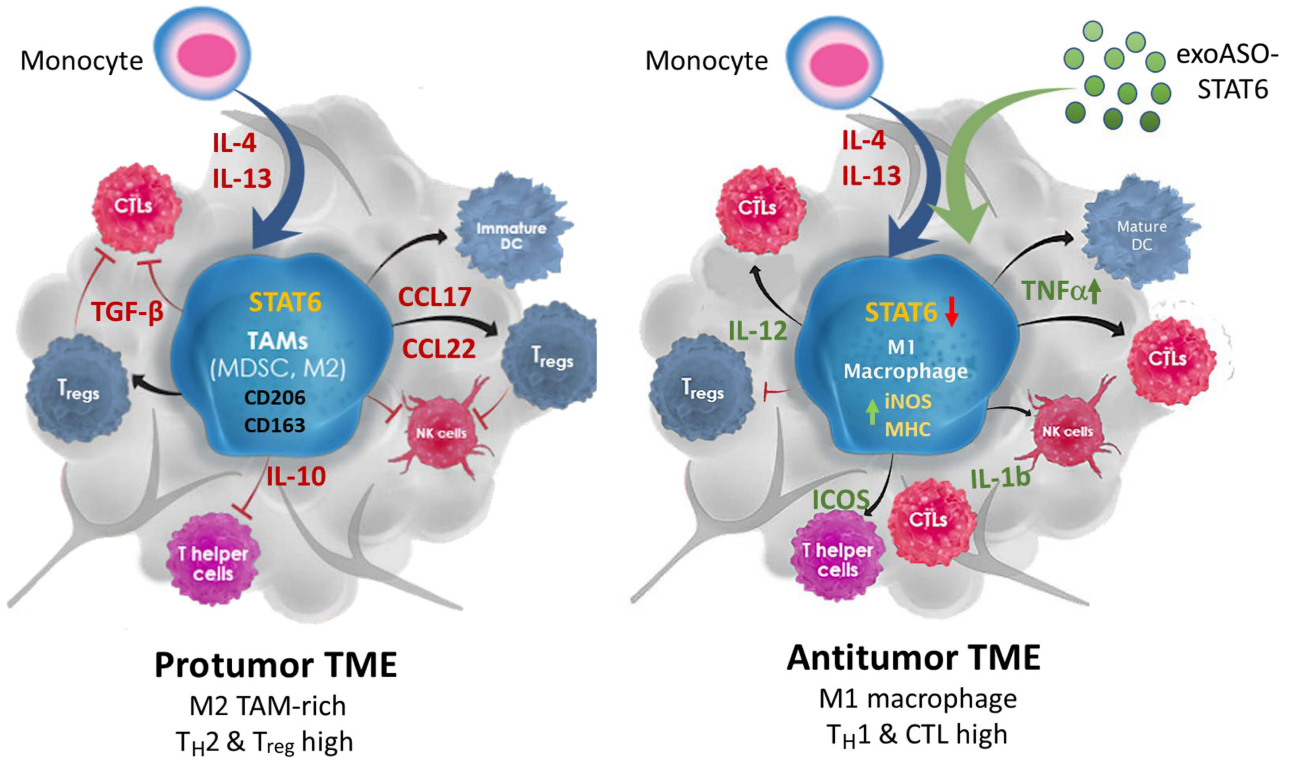

Figure 5 Model describing anti-tumor activity mediated by genetic reprogramming of TAMs by exoASO-STAT6. STAT6 expressing TAMs are critical determinants of an immunosuppressive TME by promoting recruitment of Tregs and inhibition of CD8 cytotoxic T cells. The ability of exoASO-STAT6 to selectively knock down STAT6 expression in immunosuppressive TAMs results in effective reprogramming to an M1 phenotype that promotes the induction of a cytotoxic immune response and an antitumoral TME. TH2, T helper 2; CTL, cytotoxic T lymphocytes. Reprinted from Kamerkar S, Leng C, Burenkova O, et al. Exosome-mediated genetic reprogramming of tumor-associated macrophages by exoaso-stat6 leads to potent monotherapy antitumor activity. *Sci Adv.* 2022;8(7):eabj7002. Creative Commons.[102]

are of cellular origin, they can fuse with the cell membrane and efficiently deliver inclusions into the cell. Furthermore, they can completely replicate the surface antigens of the source cell, thus avoiding complex nanomaterial engineering modifications.[103] Based on these advantages, researchers have successfully developed a variety of lipid nanoparticles based on cell membrane derivatives.

The cell membrane-derived LNVs developed to date include those of leukocyte, erythrocyte, platelet, and cancer cell origins. These vesicles exhibit distinct properties, with erythrocyte-derived, leukocyte-derived, and platelet-derived vesicles exhibiting excellent long-term circulation capabilities and frequently utilized as drug delivery vehicles to facilitate the implementation of appropriate therapeutic strategies.[104]

In addition, tumor membrane vesicles (TMVs) can be employed in tumor therapy. These vesicles are derived from cancer cells and inherently possess cancer cell membrane surface proteins and the ability to home to the tumor site; this enables TMVs to specifically bind to and be internalized by cancer cells. These abilities make TMVs highly selective delivery systems for cancer therapy.[105] Researchers have successfully designed genetically engineered cell membrane-derived nanovesicles to activate T cells and macrophages and regulate the TME based on different immune targets.[106–108] However, there has been no research related to ASO-loaded cell membrane vesicles to date; many studies have confirmed their potential as nucleic acid carriers to treat tumors, and its surface can carry cell surface antigens, a natural advantage without complex process modifications. Nanovesicles of cell membrane origin could represent an emerging promising nucleic acid delivery system in the future, with the capacity to encapsulate ASOs against RNA targets to achieve therapeutic effects on tumors.

Other ASO Delivery Systems

Although cationic liposomes can increase nonspecific cellular uptake of ASOs and promote endosomal escape through charge–charge interactions, their inherent charge toxicity and instability limit their systemic applicability for ASO

delivery.[109] Hu's team developed programmable fusion vesicles (PFVs) as a more stable alternative to cationic liposomes for systemic ASO delivery in melanoma treatment. PFVs contain a cationic lipid (DODAC) encapsulated in a PEG-ceramide component. Upon the loss of the PEG–ceramide component via an exchange-mediated process, the exposed cationic charge facilitates cell membrane binding and subsequent endocytosis.[110] This fusion liposome system not only increases the cellular uptake of ASOs but also promotes their escape from subcellular compartments into the nucleus and cytoplasm, ultimately leading to increased therapeutic activity.[111]

Human serum albumin (HSA) has been used as an ideal material for delivering nucleic acid drugs, such as ASOs and siRNA, in vivo.[112] HSA has a hydrophobic core that can be exposed during conformational changes and induce bilayer disruption or membrane fusion to achieve increased transfection efficiency.[113] Li's team synthesized and characterized lipid–albumin nanoparticles (LANs) loaded with RX-0047 to treat solid tumors. Compared with unencapsulated lipid nanoparticles, LANs demonstrated superior cellular uptake and HIF-1α mRNA downregulation efficiency, significantly inhibiting tumor growth and prolonging survival time in mice.[114]

Lipid Nanovesicles: Superior Carriers for ASO Delivery

As of March 2023, 3900 gene therapy clinical trials have been completed worldwide, and gene therapy is rapidly gaining popularity.[115] Gene editing techniques still face significant challenges and limitations. Currently, both viral and nonviral vectors (dendritic polymers, polymeric micelles, liposomes, lipid nanoparticles, solid lipid nanoparticles, and extracellular vesicles) are widely used to deliver drugs applied to nucleic acids to the appropriate sites.

Viruses can be used as vectors because they can either be modified or their inherent biology can be utilized to transport their genome to the host cell, thereby initiating the shutdown or initiation of the corresponding gene.[116] The proportion of clinically developed gene therapy products that use viral vectors remains the majority. However, viral vectors can trigger strong immune responses and safety concerns, leaving some patients unable to undergo appropriate treatment.[117] In addition, the limited nucleic acid packaging capacity of viral vectors restricts their application. In contrast, LNVs are safer and do not trigger a strong immune response. Furthermore, LNVs have a large drug-carrying capacity and can carry small-molecule drugs other than ASOs, making them well-suited for combination therapy and making their application wider.

Among nonviral vectors, dendritic polymers are also used as carriers for delivering ASOs. Dendritic polymers have a well-defined branching molecular structure, consisting mainly of a central core, repeating branching units, and a large number of terminal groups.[118] Currently, PAMAM is the most widely studied and applied typical dendrimer macromolecule. However, the cationic surface charge and the primary amine terminal group of PAMAM cause cytotoxicity problems[119] and are rapidly cleared from the plasma when administered intravenously.[120] To overcome this problem, Hu et al used dendritic PAMAM as the core. They wrapped the outer side with a pH-sensitive liposome shell for simultaneous delivery of siPD-L1 and DOX for breast cancer treatment, combining the unique advantages of the two vectors.[121] The combination of LNVs with other carriers offers new possibilities for ASO therapy.

Polymeric micelles are formed by self-assembling hydrophilic and hydrophobic amphiphilic polymers, typically ranging from 10–100 nm in size, and can extend further when encapsulating a payload.[122] Nathalie Bailly et al prepared micelles with PVP-b-PVAc and added clofazimine. In in vitro experiments, PVP-b-PVAc micelles resulted in approximately 20% drug loading in the breast cancer cell lines MCF12A and MDA-MB-231.[123] Min et al used glucosylated polyionic complex micelles loaded with antisense oligonucleotides for treating central nervous system disorders.[124] To reduce the burden of invasive ASO administration to patients, micelles were prepared from mixtures of ASO and Glu-PEG-PLL (MPA/IM) and MeO-PEG-PLL (MPA/IM) with varying amounts of glucose to prepare ligands on the PICs/Ms for GLUT1-mediated transport across the BBB. However, micelles' in vivo clearance efficiency is still unclear, and micelles are more difficult for the kidney to clear, thus increasing the risk of toxicity. In contrast, LNVs are ideal for drug delivery due to their excellent biocompatibility and higher in vivo stability. These advantages make LNVs more promising for clinical applications.

In summary, while gene therapy and nucleic acid drug delivery technologies continue to advance, it is essential to note that each carrier has unique advantages and challenges (Table 3). LNVs have emerged as particularly promising drug delivery vehicles because of their excellent biocompatibility, high drug-carrying capacity, and low immune

Table 3 Comparison of LNVs With Other Nucleic Acid Delivery Systems

Delivery Systems	Size (nm)	Component	Efficiency	Cost of Production	Advantages	Disadvantages
Viral vectors[125]	20–100	Engineered virus shell	High	High	High infection rate; high targeting; mature technology	Inflammatory and immune responses; prone to mutagenesis
Dendritic polymers[126]	1–100	Synthetic or natural elements (amino acids, sugars and nucleotides)	Medium	High	Helps encapsulate or couple multiple drug molecules while allowing controlled addition of targeted drugs on nanocarriers	High cytotoxicity; leads to cell accumulation; immunogenicity
Polymeric micelles[127]	10–100	Self-assembly of lipid monolayers in aqueous solutions	Medium-high	Low	High stability; controlled drug release	Cytotoxic; limited drug loading capacity; may trigger an immune response
Liposomes[128]	50–1000	Contains one or more lipid bilayers and an aqueous core	Medium-high	Medium	Good biocompatibility; degradability; low immunogenicity	Allergic reaction; easy oxidation degradation
Lipid nanoparticles[129]	20–100	Lipid shells surrounding the inner core, consisting of reverse micelles	High	Medium-high	Efficient delivery; high biosafety; strong loading capacity; rapid preparation process; targeted delivery	Extrahepatic targeting ability is limited and immunogenic
Solid lipid nanoparticles[130]	40–1000	A surfactant shell surrounding a core matrix composed of solid lipids	Medium	Low	Improve drug stability; control drug release rate; and improve cell membrane affinity	Limited drug loading; drug leakage and crystallization; cytotoxic
Extracellular vesicles[129]	30–1000	Natural extracellular vesicle modification	Low	High	Natural source; low immunogenicity; low toxicity; systemic circulation; targeted delivery	The technology is not yet mature, and the side effects are unknown

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response. These nanovesicles can carry multiple types of drugs and achieve targeted delivery through surface modification, a property that significantly enhances therapeutic efficacy and safety. These advantages suggest a promising future for LNVs in clinical applications and will likely stimulate the continued development of gene therapy and nucleic acid drug delivery technologies.

Concluding and Future Perspectives

The use of ASOs has proven highly effective across various fields, making it a significant milestone in nucleic acid therapy. Specific ASOs can be designed according to a patient's genomic information and delivered through LNVs to achieve personalized treatment. For example, BioNTech has developed a number of nanocarriers designed for customized therapy, focusing on using encoded mRNAs as a cancer vaccine (immuno-oncology therapy) against melanoma and breast cancer. Among them, the IVAC mutant vaccine is the most representative nanoproduct, containing encoded mRNAs individually designed for the specific expression of a single patient or a group of patient antigens.[131,132] In addition, LNVs can also be used to treat particular cancer subtypes. As mentioned above, in breast cancer, for HER2-overexpressing subtypes, ASOs designed to bind specifically to HER2 mRNA and delivered via lipid nanocarriers significantly improved therapeutic efficacy. This targeted approach allows for more precise and effective treatment, minimizing off-target effects and enhancing patient outcomes. The combination of personalized ASO design and LNV delivery systems represents a promising strategy for developing individualized cancer therapies, enabling physicians to tailor treatments to the specific genetic profile of each patient's tumor. As our understanding of cancer genomics expands, using LNVs to deliver targeted ASOs is expected to play an increasingly critical role in advancing precision medicine in oncology. This review focuses on the therapeutic applications of ASO delivery systems using LNVs in oncology, where numerous clinical trials have demonstrated their ability to significantly inhibit the expression of cancer-associated genes, thereby reducing cancer cell proliferation.

Chemical modifications have been developed to increase the efficacy, immunogenicity, and stability of ASOs, reaching the third generation. However, these chemically modified ASOs still fail to achieve active targeting of tumor sites, limiting their clinical potential in oncology. Therefore, our attention has shifted to nanomaterials, particularly LNVs. ASOs delivered via LNVs enhance stability and tumor cell uptake, specifically inhibiting the expression of oncogenes within these cells (Table 4).

Liposomes, as flagship products of LNVs, offer structural versatility, biocompatibility, biodegradability, nontoxicity, and nonimmunogenicity. However, their stability remains a significant challenge. Emerging as a new class of

Table 4 ASO-Based Cancer Therapeutics in Research

Disease	Target	ASO	Type of Nanoparticles	Ref
AML	RNR	GTI-2040	Cationic liposomes	Li et al[49]
HCC	IGF1R	CT102	Cationic liposomes	Pan et al[50]
PCa	TCTP	TCTP ASO	Cationic liposomes	Sicard et al[52]
CRCC	HIF-1α	RX-0047	pH-sensitive liposome	Yao et al[65]
GBM	miR-9	Anti-miR-9 ASO	MSC–derived Exosomes	Munoz et al[85]
BC	miR-142-3p	Anti-miR-142-3p ASO	MSC–derived Exosomes	Naseri et al[87]
CRCC	miR-221	Anti-miRNA-221 ASO	MSC–derived Exosomes	Han et al[89]
CRCC	miR-146b-5p	Anti-miR-146b-5p ASO	MSC–derived Exosomes	Yu et al[90]
AML/ BC	miR-125b	Anti-miR-125b ASO	RBCEVs	Usman et al[92]
AML	FLT3-ITD and miR-125b	FLT3-ITD ASO and Anti-miR-125b ASO	RBCEVs	Chen et al[95]
CRCC and HHC	STAT6	STAT6 ASO	HEK 293-derived Exosomes	Kamerkar et al[102]
Melanoma	Bcl-2	G3139	Fusogenic liposome	Hu et al[111]
Lung Cancer	miR-21	Anti-miR-21 ASO	SLN	Shi et al[69]
Solid Tumors	HIF-1α	RX-0047	LAN	Li et al[114]

Abbreviations: AML, Acute myelogenous leukemia, HCC, Hepatocellular carcinoma, PCa, Prostate cancer, CRC, Colorectal Carcinoma, GBM, Glioblastoma multiforme, BC, Breast cancer, RNR, Ribonucleotide reductase, IGF1R, Insulin-like Growth Factor type 1 Receptor, TCTP, Translational Controlled Tumour Protein, HIF-1α, Hypoxia-inducible Factor 1α, FLT3-ITD, Internal tandem duplications of the FLT3 gene, STAT6, Signal Transducer and Activator of Transcription 6, Bcl-2, B-cell lymphoma-2, MSC, Mesenchymal Stem Cell, RBCEVs, Red blood cells extracellular vesicles, SLN, Solid lipid nanoparticles, LAN, Lipid-albumin nanoparticle.

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nanocarriers, EVs present lower immunogenicity, better biocompatibility than liposomes, and the ability to target specific cells and tissues. Despite these advantages, the production, characterization, and functionalization of EVs require continuous improvement, and the encapsulation efficiency of ASOs remains an urgent issue.

The development of CRISPR-Cas9 and RNA editing technology, in combination with LNVs, has opened new avenues for precise gene therapy. In collaborative applications, LNVs play a crucial role in encapsulating and safeguarding the nucleic acid sequences required for CRISPR/Cas9 components (such as Cas9 mRNA and sgRNA) or RNA editing. This protective function prevents their degradation in the circulatory system, thereby increasing their stability and delivery efficiency within the body. Zhang et al combined pCas9/sgRNA with protamine (PS), coated the complex with cationic liposomes to protect the plasmid from nucleic acid degradation and added DSPE-PEG modification to obtain PEGylated lipid nanoparticles (PLNPs). They then injected pCas9/gPLK-1 PLNPs into the tumor, achieving a remarkable 67% inhibition rate of tumor growth.[133] Furthermore, surface modification of LNVs enables targeted delivery to various tissues, significantly expanding the application scope of CRISPR–Cas9 and RNA editing tools. By functionalizing the surface of LNVs with specific ligands or antibodies, researchers can direct the gene-editing machinery to the desired cells or organs, thereby increasing the precision and efficacy of the treatment. The synergistic effect of CRISPR–Cas9, RNA editing technology, and LNVs provides a safer and more effective platform for the gene therapy of human diseases.

While the potential of ASOs and their delivery systems is promising, critically evaluating the current limitations and challenges is crucial. One significant concern is the high cost and complexity of developing and producing these advanced delivery systems, which may limit their accessibility and widespread adoption in clinical settings. Additionally, the long-term safety and potential off-target effects of ASOs and their delivery vehicles need thorough investigation, as unintended interactions with nontarget cells or tissues could lead to adverse effects.

The versatility, flexibility, and short development cycle of ASOs position them as significant competitors within the field of gene therapy. However, despite chemical modifications, ASOs have the potential to trigger an immune response and cause adverse reactions. They may also bind to nontarget genes, resulting in unintended gene silencing or activation, which may induce side effects. Therefore, it is essential that ASOs undergo long-term clinical safety evaluation and further research to ensure their efficacy and safety. In recent years, based on encouraging results obtained from using LNVs for nucleic acid therapy, there is a growing expectation that ASOs will soon overcome the limitations of the current delivery systems. ASO therapy based on LNVs is expected to represent a new generation of personalized nanomedicines that have the potential to change the face of oncological treatment by improving circulation time, cellular uptake efficiency, and promoting endosomal escape. The continuous development of novel nanoplatforms and target development technologies, in conjunction with advances in clinical validation, is anticipated to overcome the limitations of the current delivery system. LNV-based ASO therapeutics are projected to become a new generation of personalized nanomedicines with the potential to transform the landscape of tumor treatment, and there is a high degree of confidence in their clinical translation.

Abbreviations

mRNA, messenger RNA; ASOs, antisense oligonucleotides; siRNAs, small interfering RNAs; miRNAs, microRNAs; 3′ UTR, 3′untranslated region; AMOs, antisense miRNA oligonucleotides; PS, phosphorothioate; 2’-OMe, 2’-O-methyl; 2’-MOE, 2’-O-methoxy-ethyl; 2’-F, 2’-fluoro; AML, acute myeloid leukemia; DOC-PEI, deoxycholate-polyethyleneimine coupling; aCD33, anti-CD33; IGF1R, insulin-like growth factor type 1 receptor; HCC, hepatocellular carcinoma; TCTP, Translationally controlled tumor protein; CRPCa, castration-resistant prostate cancer; HIF-1α, hypoxia-inducible factor 1α; anti-DR5, couple death receptor 5-specific antibodies; EVs, Extracellular vesicles; MSCs, mesenchymal stem cells; GBM, glioblastoma multiforme; hUC-MSC, human umbilical cord mesenchymal stem cell; RBCEV, red blood cell extracellular vesicle; FLT3, FMS-like tyrosine kinase 3; TAMs, Tumor-associated macrophages; TME, tumor microenvironment; TMVs, tumor membrane vesicles; PFV, programmable fusion vesicle; SLN, Solid lipid nanoparticles; HSA, human serum albumin; LAN, lipid-albumin nanoparticle; LNP, Lipid nanovesicle.

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Author Contributions

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

Disclosure

The authors declare no conflicts of interest in this work.

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