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REVIEW

Targeted Delivery of Nucleic Acid Therapeutics: Emerging Carriers and Applications in Common Metabolic and Inflammatory Diseases

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Abstract: Recent advances in genomics and molecular biology have accelerated the development of nucleic acid-based therapeutics. To optimize their clinical efficacy, various delivery systems—including lipid nanoparticles, polymeric nanomaterials, inorganic nanoparticles (NPs), extracellular vesicles (EVs), viral vectors, protein carriers, and nucleic acid conjugates—have been explored to improve stability, cellular uptake, and bioavailability. This review provides an overview of the current state of nucleic acid therapeutics, focusing on their classification, delivery strategies, and applications in metabolic and inflammatory diseases. Emphasis is placed on the mechanisms of action, targeted delivery approaches, and the existing challenges these systems face. Literature was sourced from PubMed and Web of Science, relevance to the topic, and publication timeline (2020–2025). The aim is to propose insights into the optimization of nucleic acid drugs and the development of novel delivery systems, offering new perspectives for the treatment of complex diseases.

Keywords: nucleic acid therapeutics, delivery systems, metabolic diseases, inflammatory diseases

Introduction

Nucleic acid therapeutics, encompassing both naturally occurring nucleic acids (DNA and RNA) and synthetically engineered molecules with nucleic acid structures, represent a promising modality for disease intervention through gene silencing, replacement, and editing.[1] These agents have garnered increasing attention due to their rapid target identification, high therapeutic efficiency, low toxicity, strong specificity, and relatively high success rate in drug development.[2] Although the development of nucleic acid–based medicines has spanned several decades, the sudden outbreak of COVID-19 and the emergency authorization of messenger RNA (mRNA) vaccines markedly accelerated interest in this field, bringing nucleic acid therapeutics to the forefront of biomedical research.[3] The 2023 Nobel Prize in Physiology or Medicine, awarded for the discovery of nucleoside base modifications that reduce mRNA immunogenicity, further catalyzed the exploration and clinical translation of nucleic acid drugs.[4] Currently, various modalities-including antisense oligonucleotide (ASO), mRNA, microRNA (miRNA), small interfering RNA (siRNA), and small activating RNA (saRNA)-are under active investigation, with ongoing studies continually refining their classification and mechanisms of action.[5,6]

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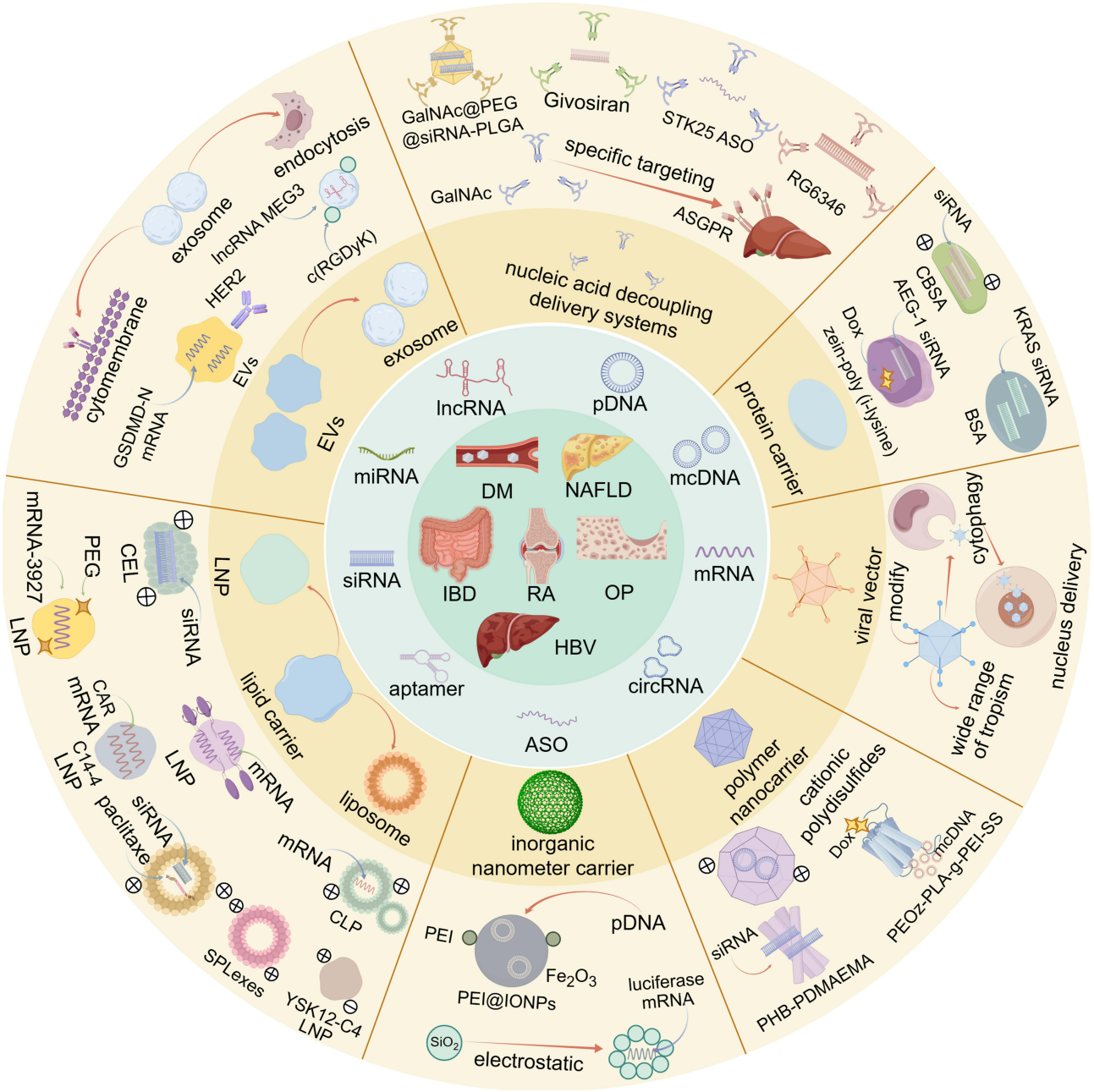
Graphical Abstract



Despite their tremendous potential, the clinical translation of nucleic acid therapeutics is hindered by several barriers, such as inherent instability, negative charge, limited tissue targeting, and difficulty in crossing biological barriers.[7] To overcome these challenges, researchers have developed strategies including chemical modification of nucleic acids and targeted molecular engineering to enhance stability and delivery efficiency while minimizing immunogenicity.[8,9] Notably, the emergence of advanced delivery vectors has significantly improved the ability of nucleic acid drugs to traverse biological barriers, thereby enhancing their in vivo stability and therapeutic efficacy.[9]

To systematically evaluate the current landscape of "targeted delivery of nucleic acid therapeutics: emerging carriers and applications in common inflammatory and metabolic diseases", we followed a structured approach for literature

retrieval and selection to ensure comprehensiveness and representativeness. A detailed search of PubMed and Web of Science was conducted up to November 2025, using a combination of core subject terms (eg, "nucleic acids") and relevant free-text keywords to balance sensitivity and specificity. Eligible studies included randomized controlled trials, authoritative guidelines, large observational studies, and high-impact review articles, with a focus on nucleic acid drug delivery and therapeutic applications. Only English-language publications from 2020 to 2025 were considered. Screening was performed in two phases: (1) preliminary selection based on titles and abstracts, followed by (2) full-text assessment of potentially relevant articles. Special attention was given to methodological rigor and the clinical relevance of findings. Given the narrative nature of this review, we adopted thematic analysis to synthesize existing evidence, aiming to delineate the conceptual framework, prevailing consensus, and remaining controversies in the field.

Although previous studies have reviewed nucleic acid delivery systems—including extracellular vesicles and other emerging carriers-the present review provides a more systematic and comprehensive overview. It covers a broad spectrum of nucleic acid drug types and delivery platforms, examines physicochemical properties, mechanisms of action, advantages and limitations of different nucleic acid therapeutics, and compares the performance of diverse delivery systems. Moreover, it discusses the synergistic applications of nucleic acid therapeutics and delivery vehicles in common metabolic and inflammatory diseases. Through this integrated analysis, the review aims to offer a forward-looking perspective to guide future research and clinical translation of nucleic acid therapeutics.

Classification of Nucleic Acid Drugs

Nucleic Acid Drugs with Coding Potential

Plasmid DNA (pDNA)

Plasmid DNA (pDNA), a non-essential extrachromosomal macromolecule found within cells, can transfer between different hosts and replicate in suitable biological environments, making it an important platform for gene therapy.[6,10] Owing to its intrinsic negative charge, pDNA can form stable electrostatic complexes with positively charged delivery vectors or be directly encapsulated within carriers.[11,12] Studies have shown that the likelihood of pDNA unintentionally integrating into the host genome is extremely low.[13] Furthermore, the development of pDNA-based vaccines has progressed considerably due to their high stability, cost-effectiveness, and favorable safety profile.[14] However, efficient delivery remains challenging because pDNA must enter the cell nucleus to initiate gene expression (Figure 1).[15]

Through genetic engineering, pDNA can be designed to carry therapeutic genes and delivered to target cells to enable gene transfection. This process can elicit protective immune responses or provide functional proteins for treating various diseases. For example, pDNA encoding vascular endothelial growth factor (VEGF), glial cell line–derived neurotrophic factor, or hepatocyte growth factor (HGF) has been shown to significantly improve cerebral ischemia outcomes in rodent models.[16,17] Moreover, pDNA constructs targeting amyloid-β or myelin basic protein have demonstrated immunogenic potential, suggesting utility in vaccines for Alzheimer's disease (AD) and multiple sclerosis.[17]

In the field of oncology, bifunctional fusion pDNA complexes expressing catalase and the photosensitive protein KillerRed, together with star-shaped cationic poly(disulfide)s containing β-cyclodextrin cores, were found to alleviate tumor hypoxia, remodel the tumor microenvironment, and markedly inhibit tumor growth.[18] Additionally, pDNA encoding soluble FMS-like tyrosine kinase-1 has exhibited potent anti-angiogenic and anti-tumor efficacy in colorectal cancer (CRC).[19] pDNA-mediated suicide gene delivery has also demonstrated therapeutic value in ovarian cancer mouse models, significantly suppressing tumor progression.[20,21]

Minicircle DNA (mcDNA)

As another class of coding nucleic acid therapeutics, minicircle DNA (mcDNA) is generated by removing all bacterial backbone sequences while retaining only the gene expression cassette. This structural refinement results in a smaller molecular size, reduced immunogenicity, and improved nuclear delivery efficiency, enabling direct encapsulation and delivery via lipid nanoparticles (LNPs).[22–25] These advantages make mcDNA highly suitable for applications requiring efficient gene expression, including induced pluripotent stem cell (iPSC) generation and cancer therapy.[26] For example, Jia et al utilized mcDNA encoding reprogramming factors to induce human adipose-derived stem cells (ASCs) into iPSCs, achieving significantly higher reprogramming efficiency compared with pDNA-based approaches.[27] mcDNA has

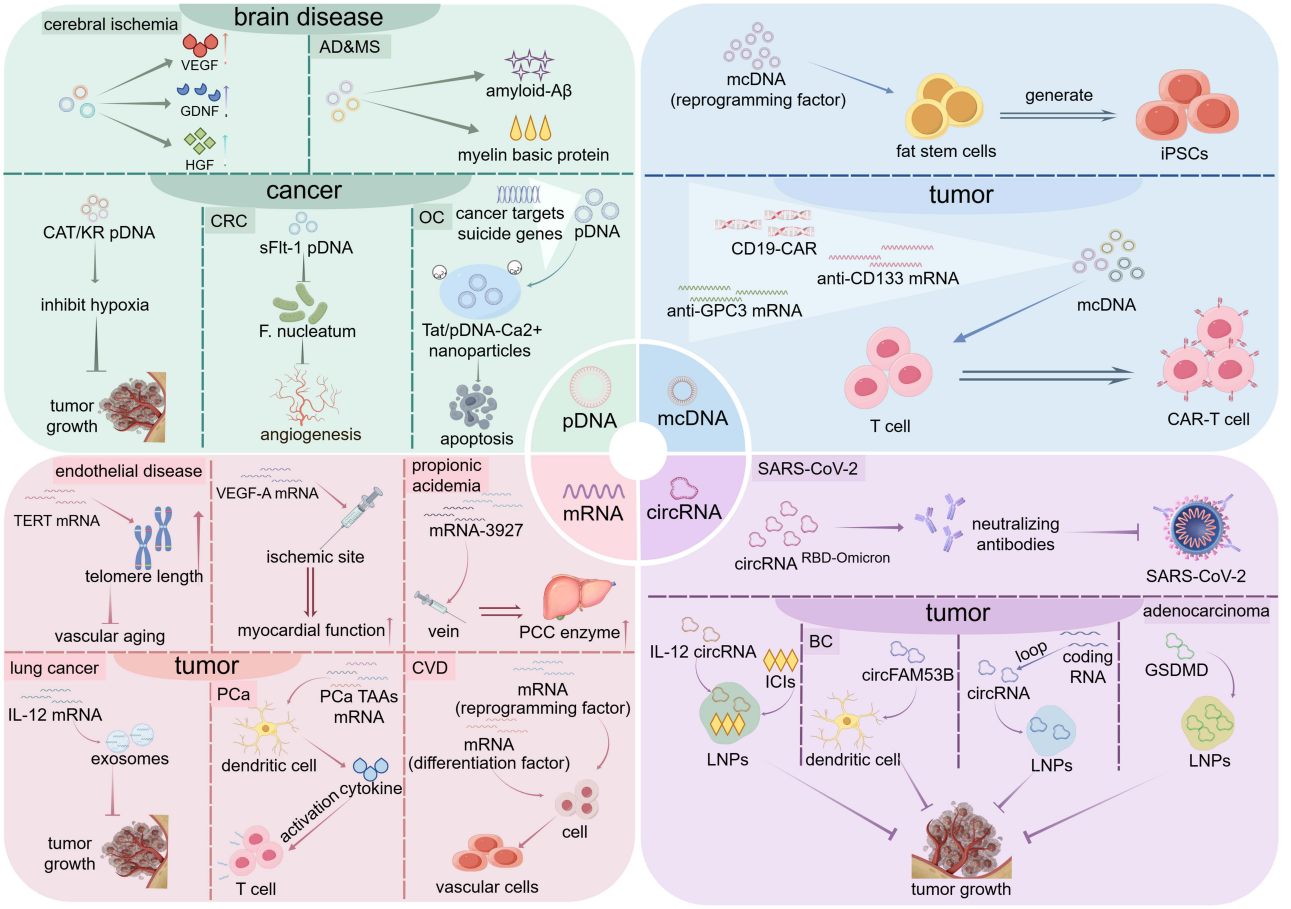


Figure 1 Nucleic acid therapeutics with protein-coding potential-including pDNA, mcDNA, mRNA, and circRNA-exert therapeutic effects across diverse diseases by modulating cellular pathways involved in apoptosis and immune regulation.

Notes: Upward and downward arrows indicate increases and decreases, respectively; T-shaped arrows denote inhibition or negative regulation; other arrows represent associations or process flow.

Abbreviations: VEGF, vascular endothelial growth factor; GDNF, glial cell line-derived neurotrophic factor; HGF, hepatocyte growth factor; AD, Alzheimer's disease; MS, multiple sclerosis; CAT, catalase; KR, KillerRed; CRC, colorectal cancer; sFlt-1, soluble FMS-like tyrosine kinase-1; OC, ovarian cancer; iPSCs, induced pluripotent stem cells; CAR, chimeric antigen receptor; GPC3, glypican-3; TERT, telomerase reverse transcriptase; VEGF-A, vascular endothelial growth factor-A; PCC, propionyl-coenzyme A carboxylase; IL, interleukin; PCa, prostate cancer; TAAs, tumor-associated antigens; circRNA$^{RBD\text{-}Omicron}$, circRNA vaccine expressing the spike protein trimeric receptor-binding domain; ICIs, immune checkpoint inhibitors; LNPs, lipid nanoparticles; BC, breast cancer; GSDMD, gasdermin D.

also been widely applied in chimeric antigen receptor (CAR)-T cell engineering.[28] Using electroporation, Wang et al introduced anti-CD133 and anti-glypican-3 single-chain variable fragments into mcDNA constructs, enabling the efficient and safe production of bispecific CAR-T cells that demonstrated markedly enhanced anti-tumor activity in liver cancer models.[29] Similarly, Ye et al generated CAR-T cells using mcDNA and confirmed their potent tumor-suppressive effects through in vivo studies (Figure 1).[28]

Messenger RNA (mRNA)

mRNA can be synthesized through in vitro transcription (IVT) followed by additional processing steps.[30–32] Unlike DNA therapeutics, which require nuclear delivery and pose a risk of genomic integration, mRNA functions exclusively in the cytoplasm, offering a superior safety profile.[33,34] However, mRNA is highly susceptible to degradation by nucleases, necessitating encapsulation within protective delivery vectors to enhance stability.[35,36] Moreover, the transient expression nature of mRNA after cellular uptake often requires repeated dosing, which may increase the risk of adverse events (Figure 1).[37]

Despite these challenges, mRNA holds immense promise in infectious disease vaccines, cancer therapy, and protein replacement strategies. Compared with conventional vaccines, mRNA-based formulations enable rapid development and

rapid production of antigenic proteins, making them particularly advantageous in responding to emerging infectious diseases.[38,39] mRNA therapeutics leverage endogenous cellular translation machinery to rapidly produce diverse functional proteins.[38] For example, mRNA encoding human telomerase reverse transcriptase has been shown to elongate telomeres and delay senescence marker expression, offering potential benefits in vascular aging–related disorders.[39,40] Direct intramyocardial injection of mRNA encoding VEGF-A following myocardial infarction significantly improves cardiac function and survival without inducing toxic effects, and these findings have progressed to clinical trials.[41–43] Similarly, mRNA-3927, a dual mRNA therapy designed for propionic acidemia, delivers propionyl-coenzyme A carboxylase(PCC) α- or PCC β-encoding mRNA via LNPs to restore PCC activity in the liver and is currently in mid-stage clinical development.[44]

In cancer therapy, exosome-loaded mRNA encoding interleukin(IL)-12 has been shown to sustain local IL-12 expression in lung tissue, enhance immune activation and memory responses, and effectively inhibit tumor progression.[45] mRNA vaccines that encode tumor-associated antigens also demonstrate strong therapeutic potential; Xu et al developed a four-antigen prostate cancer (PCa) mRNA vaccine that promoted dendritic cell cytokine release, enhanced T cell activation, and produced robust cytotoxic responses in PCa mouse models.[46]

mRNA technology is also being exploited for cellular reprogramming applications. mRNA-induced iPSCs exhibit greater consistency and stability compared to retroviral-derived counterparts.[47] This approach additionally accelerates differentiation; for instance, mRNA delivery of reprogramming or lineage-specific drivers has successfully generated cardiovascular cells in vitro, underscoring its promise in cardiovascular disease (CVD) therapy.[39]

Circular RNA (circRNA)

Circular RNA (circRNA) is a covalently closed-loop RNA generated through back-splicing of exons or introns in eukaryotic cells.[48] Lacking both a 5' cap and a 3' poly(A) tail, circRNA exhibits strong resistance to exonuclease-mediated degradation, resulting in exceptional stability and prolonged half-life.[49,50] These features make circRNA an attractive modality for sustained therapeutic protein expression;[37,51] however, circRNA is inefficiently internalized by cells and is rapidly cleared in vivo, necessitating effective delivery systems (Figure 1).[52]

Recent studies highlight the dual role of circRNA in cancer—both as a regulatory molecule and as a potent therapeutic agent.[53] For example, circFAM53B, highly expressed in the cytoplasm of breast cancer (BC) cells, enhances cytotoxic T lymphocyte differentiation and stimulates adaptive antitumor immunity.[54] Intravenous infusion of autologous dendritic cells loaded with circFAM53B significantly inhibited tumor growth in BC xenograft models.[54,55] Feng et al engineered a mitochondria-targeting gasdermin D (GSDMD) circRNA construct for adenocarcinoma therapy. Delivered intraperitoneally via LNPs, this therapy achieved robust tumor ablation in vivo.[55]

In the field of immunotherapy, Xu et al encapsulated IL-12 circRNA into LNPs and administered it either intratumorally or intratracheally; combined with immune checkpoint inhibitors, this strategy induced stable tumor regression.[37] Li et al demonstrated that synthetic circularization of linear RNA significantly enhances vaccine stability when delivered through LNPs.[56] Intramuscular administration of these circRNA vaccines produced strong innate and adaptive immune responses in multiple tumor models, while systemic prophylactic delivery further enhanced immunogenicity.[56] Qu et al developed a circRNA vaccine expressing the Omicron trimeric receptor-binding domain, which elicited sustained antigen expression and durable immunity in mice and rhesus macaques, demonstrating protective potential against SARS-CoV-2 variants.[57]

Nucleic Acid Drugs with Uneven Coding Potential

Antisense Oligonucleotide (ASO)

ASOs are short nucleic acid sequences, typically 15–25 nucleotides in length, generated through antisense technology.[58–60] They hybridize complementarily to specific RNA or DNA sequences.[61] In addition to vector-based delivery, multiple chemical modification strategies have been developed to protect ASOs from nuclease degradation, enhance molecular stability, and reduce immunogenicity.[62–64] These modified ASOs possess moderate molecular size and generally do not require delivery vectors, thereby lowering production costs and enabling efficient distribution to target tissues through diverse administration routes (Figure 2).

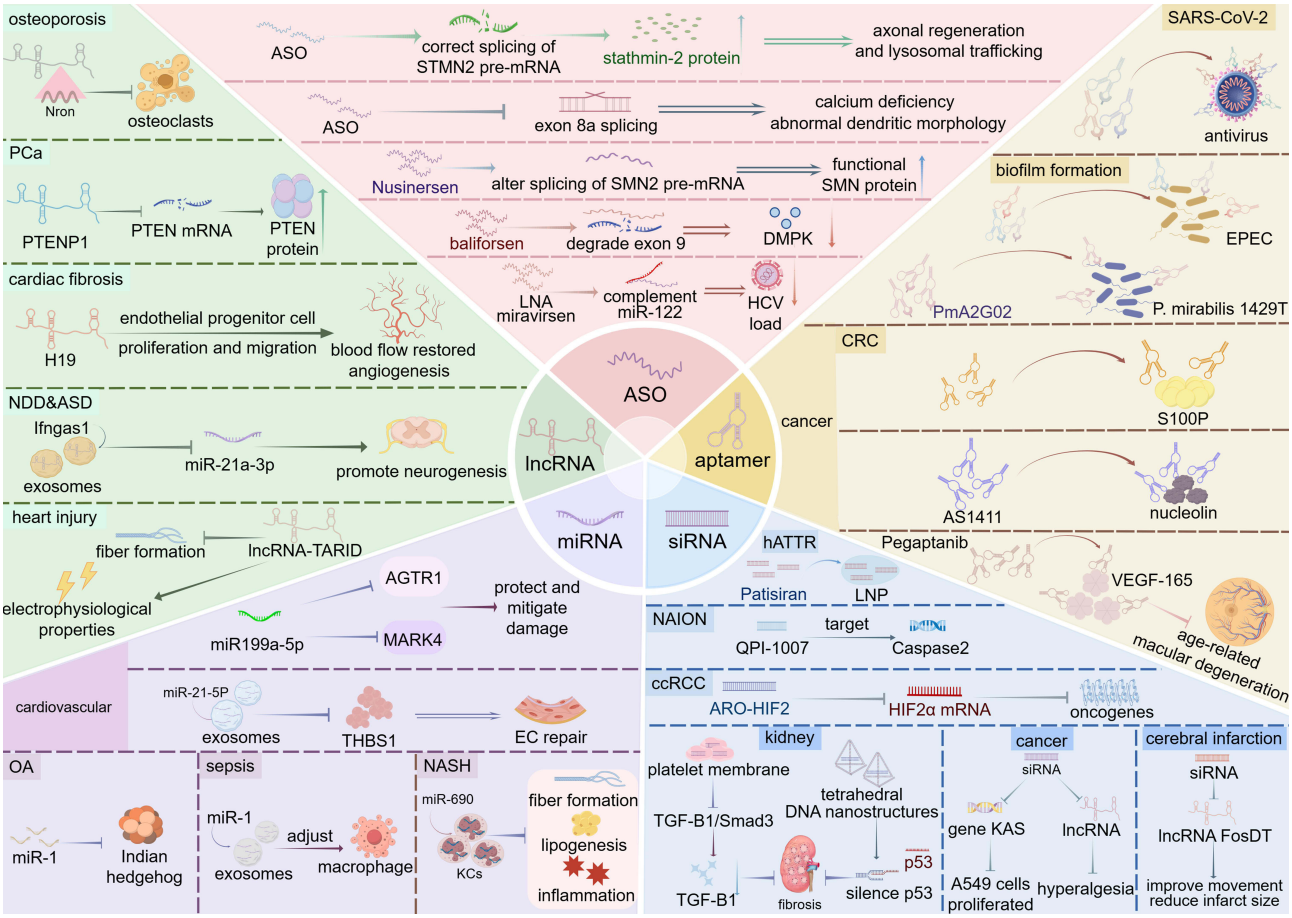


Figure 2 Non-coding nucleic acid therapeutics-including ASOs, aptamers, siRNAs, miRNAs, and lncRNAs-modulate protein expression through direct or indirect mechanisms such as regulating mRNA splicing, translation, or post-transcriptional processing, thereby influencing disease progression.

Notes: Upward and downward arrows indicate increases and decreases, respectively; T-shaped arrows denote inhibition or negative regulation; other arrows represent associations or process flow.

Abbreviations: PCa, prostate cancer; NDD, neurodegenerative diseases; ASD, autism spectrum disorder; STMN2, survival motor neuron 2; mRNA, messenger RNA; SMN2, survival motor neuron 2; DMPK, DM1 protein kinase; HCV, hepatitis C virus; EPEC, enteropathogenic E. coli; P. mirabilis 1429T, against Proteus mirabilis 1429; CRC, colorectal cancer; VEGF, vascular endothelial growth factor; AGTR1, Angiotensin II receptor type 1; MARK4, microtubule affinity regulating kinase 4; THBS1, Thrombospondin-1; EC, endothelial cell; OA, osteoarthritis; NASH, non-alcoholic steatohepatitis; KCs, Kupffer Cells; hATTR, hereditary transthyretin amyloidosis; LNP, lipid nanoparticles; NAION, non-arteritic anterior ischemic optic neuropathy; ccRCC, clear cell renal cell carcinoma; TGF-β1, transforming growth factor-β1.

ASO therapeutics regulate gene expression by binding to target mRNAs and have been extensively explored in various diseases.[65] For example, Timothy syndrome type 1 (TS1) is caused by a gain-of-function mutation in exon 8A. Chen et al inhibited exon 8A splicing using ASOs, successfully rescuing depolarization-induced calcium defects and abnormal dendritic morphology in rats transplanted with human TS1 cortical organoids following a single intrathecal injection.[66] Baughn et al delivered ASOs into neuronal cells using electroporation or transfection reagents and injected them into the ventricles of TDP-43 proteinopathy model mice, correcting STMN2 pre-mRNA splicing defects, restoring stathmin-2 protein levels, and enhancing axon regeneration and lysosomal transport.[67]

Several ASO drugs have advanced into clinical safety trials. Nusinersen, the first FDA-approved ASO for treating spinal muscular atrophy (SMA),[68] modulates survival motor neuron 2 precursor mRNA splicing via intrathecal injection to increase functional SMN protein expression, substantially improving SMA symptoms.[69] Baliforsen, an ASO targeting exon 9 of DMPK RNA for type 1 myotonic dystrophy, promotes RNase H1-mediated degradation through subcutaneous administration and is currently under clinical evaluation.[70] Miravirsen, one of the earliest miRNA-based ASO candidates, inhibits the 5' region of miR-122 and effectively reduces hepatitis C virus load and hepatic cholesterol accumulation in clinical trials with minimal adverse events.[71,72]

Nucleic Acid Aptamers (Aptamers)

Aptamers are single-stranded oligonucleotides capable of binding target molecules with high specificity.[73] They are selected using Systematic Evolution of Ligands by Exponential Enrichment.[74,75] Compared with antibodies, aptamers exhibit greater thermal stability,[76] a broader spectrum of target recognition,[77] smaller molecular size, and lower production cost,[69,70] and typically show minimal immunogenicity.[78,79] Owing to their high bioavailability and target specificity, aptamers can function as therapeutic agents or as ligands to modify NPs for drug delivery (Figure 2).[80,81]

Clinically, aptamers effectively combat biofilm-related infections.[82] Oroh et al demonstrated that six aptamers inhibited biofilm formation by enteropathogenic E. coli by reducing bacterial motility and downregulating transcripts of biofilm-associated genes.[83] Elumalai et al showed that aptamer PmA2G02 exerted anti-biofilm activity against Proteus mirabilis 1429 by suppressing bacterial motility, viability, and adhesion.[84] Aptamers also hold considerable promise in viral diagnostics and therapy, particularly for recognizing various SARS-CoV-2 domains, enabling rapid detection and antiviral intervention.[84,85]

In cancer therapy, aptamers exhibit strong therapeutic potential.[74,86] For instance, an aptamer targeting the over-expressed S100P protein in CRC inhibited CRC cell proliferation in vitro and, upon subcutaneous injection, reduced tumor growth in CRC xenograft mice.[87] To date, only a limited number of aptamer-based therapeutics have entered clinical trials.[6] Pegaptanib, the first FDA-approved RNA aptamer, treats age-related macular degeneration by binding VEGF-165 to inhibit angiogenesis.[88–90] AS1411, the first DNA aptamer to reach cancer clinical trials, targets nucleolin and suppresses tumor proliferation by modulating multiple signaling pathways.[91]

Small Interfering RNA (siRNA)

siRNA is a double-stranded RNA molecule that forms the RNA-induced silencing complex in the cytoplasm.[92–94] Short hairpin RNAs, synthesized in the nucleus, are transported to the cytoplasm and processed into active siRNAs via RNA interference.[95] Due to their large size, negative charge, and hydrophilicity, siRNAs cannot freely diffuse across cell membranes and therefore require delivery systems or chemical modification for efficient cellular uptake (Figure 2).[96]

Clinically, intravenously administered siRNAs are predominantly filtered by the kidneys, enabling accumulation in renal tissues and providing a therapeutic strategy for kidney diseases.[97] For example, Fei et al used nanoparticle systems camouflaged with platelet membranes to deliver siRNA to injured renal tissues, successfully inhibiting the transforming growth factor-β1 (TGF-β1)/Smad3 pathway, reducing TGF-β1 expression, and alleviating renal fibrosis and acute injury in mice.[98] Li et al employed tetrahedral DNA nanostructures modified with three cholesterol molecules to effectively deliver p53 siRNA to renal tubules, preventing acute kidney injury.[99]

siRNA applications extend to cancer and rare disease therapy.[100,101] siRNA targeting the KAS gene effectively inhibited A549 tumor cell proliferation.[102] Sun et al reduced cancer-induced bone pain in rats through intrathecal siRNA delivery targeting specific long non-coding RNA (lncRNA).[103] In advanced clear cell renal cell carcinoma, the siRNA therapeutic ARO-HIF2 suppresses hypoxia-inducible factor-2α mRNA, thereby interfering with oncogenic signaling, although its efficacy and safety require further study.[104] Patisiran, the first FDA-approved siRNA drug, uses LNPs for hepatic delivery to treat hereditary transthyretin amyloidosis.[105] For ocular diseases, the siRNA drug QPI-1007 targets the Caspase-2 gene via intravitreal injection and yields superior visual recovery in non-arteritic anterior ischemic optic neuropathy compared with surgical treatment.[106] Mehta et al found that siRNA-mediated knockdown of lncRNA FosDT significantly improved motor function and reduced cerebral infarct volume in rodent models of transient middle cerebral artery occlusion.[107]

MicroRNA (miRNA)

miRNAs are non-coding RNAs that typically suppress translation of target mRNAs,[108] although in certain contexts they can enhance translation.[109] They play essential roles in cell differentiation, proliferation, and gene expression regulation[110,111] and are implicated in cancer progression and therapeutic resistance.[112] However, the clinical translation of miRNA therapeutics remains limited due to low specificity, suboptimal biosafety, and poor transfection efficiency, restricting their broader application (Figure 2).[113,114]

In disease intervention, miRNAs regulate protein expression either by repressing[115] or promoting[116] target mRNA expression. Hu et al demonstrated that miR-21-5p in endothelial progenitor cell-derived exosomes inhibits Thrombospondin-1, thereby promoting endothelial cell repair.[117] miR-199a-5p shows therapeutic potential in cardiac disease by targeting AGTR1 and MARK4, reducing oxidative damage, and improving cardiac function after myocardial infarction.[118] Chen et al delivered miR-199a-5p to ischemic myocardium using P-MSN/miR-199a-5p nanoparticles, significantly reducing myocardial injury.[118] Li et al delayed osteoarthritis(OA) progression in rats through intra-articular injection of miR-1 mimics.[119] Zheng et al reported that miR-150-5p in mesenchymal stem cell–derived exosomes modulates macrophage plasticity, thereby reducing inflammation in mouse sepsis models.[120] Gao et al demonstrated that nanoparticle-mediated miR-690 delivery in non-alcoholic steatohepatitis (NASH) mice inhibited the expression of fibrosis-related genes.[121]

Long Non-Coding RNA (lncRNA)

Recent studies have revealed that lncRNAs play essential roles in regulating gene and protein expression, exerting effects on both proximal and distal genomic loci.[122,123] They hold considerable promise for diagnostics and therapeutics,[122,123] and functional lncRNA motifs may offer reduced toxicity compared with full-length transcripts.[124] Recent advances in LNP systems with enhanced surface cationic charge have improved the electrostatic condensation of lncRNAs, thereby reducing cytotoxicity and increasing cellular uptake efficiency (Figure 2).[125]

Jin et al identified lncRNA Nron as a key negative regulator of bone resorption, with its functional motif effectively reversing bone loss while producing minimal side effects.[124] PTENP1, a pseudogene of the tumor suppressor PTEN, enhances PTEN protein levels by blocking miRNA-mediated degradation, thereby suppressing PCa progression.[126] Huang et al demonstrated that endothelial progenitor cells overexpressing lncRNA H19 exhibited enhanced proliferation, migration, and tube formation in a mouse ischemic limb model, promoting neovascularization.[127] Fu et al reported that human adipose-derived mesenchymal stem cell–derived exosomes containing lncRNA Ifngas1 inhibit miR-21a-3p, thereby improving inflammatory conditions in the central nervous system and promoting neurogenesis.[128] Zhu et al showed that lncRNA-TARID increases Tcf21 expression, reduces myocardial fibrosis, and improves cardiac function in models of heart injury.[129]

Nucleic Acid Drug Delivery Vectors

Lipids

Over the past several decades, numerous lipid-based systems have been developed for intracellular nucleic acid delivery.[130] These lipids can self-assemble into a variety of nanostructures—including micelles, liposomes, and LNPs—that play critical roles in ensuring the safety and efficacy of nucleic acid therapeutics.[9] Among them, LNPs are spherical carriers approximately 100 nm in diameter and are typically composed of cationic or ionizable lipids, cholesterol, helper lipids, and polyethylene glycol (PEG)–modified lipids.[131,132] Due to their simple manufacturing processes, low systemic immunogenicity, and high RNA-encapsulation efficiency, LNPs are considered one of the most advanced and clinically promising vectors for nucleic acid delivery.[132–136] For example, Koeberl et al used PEGylated LNPs to encapsulate mRNA-3927, reducing phagocytic uptake and enhancing targeted delivery to hepatocytes.[44] Variations in lipid structure result in distinct interactions with cellular membranes, which in turn influence delivery efficiency and therapeutic performance,[137–141] highlighting the importance of designing disease-specific delivery systems (Figure 3).[37]

Ionizable lipids are characterized by their hydrophilic head groups containing ionizable amines,[142,143] linkages,[143,144] and hydrophobic tail chains.[143,144] These structural features are crucial for mRNA encapsulation within LNPs and for promoting endosomal escape into the cytoplasm.[145] Under physiological pH, these lipids remain neutral but ionize and acquire a positive charge in acidic environments, enhancing nucleic acid binding efficacy.[35] Furthermore, ionizable lipids can disrupt endosomal membranes to facilitate escape,[146] enabling safe and effective systemic nucleic acid delivery.[147] Nelson et al demonstrated that LNPs formulated with ionizable phospholipids significantly enhanced mRNA endosomal escape and transfection efficiency, promoting bone formation in a murine tibial fracture model.[148] Billingsley et al

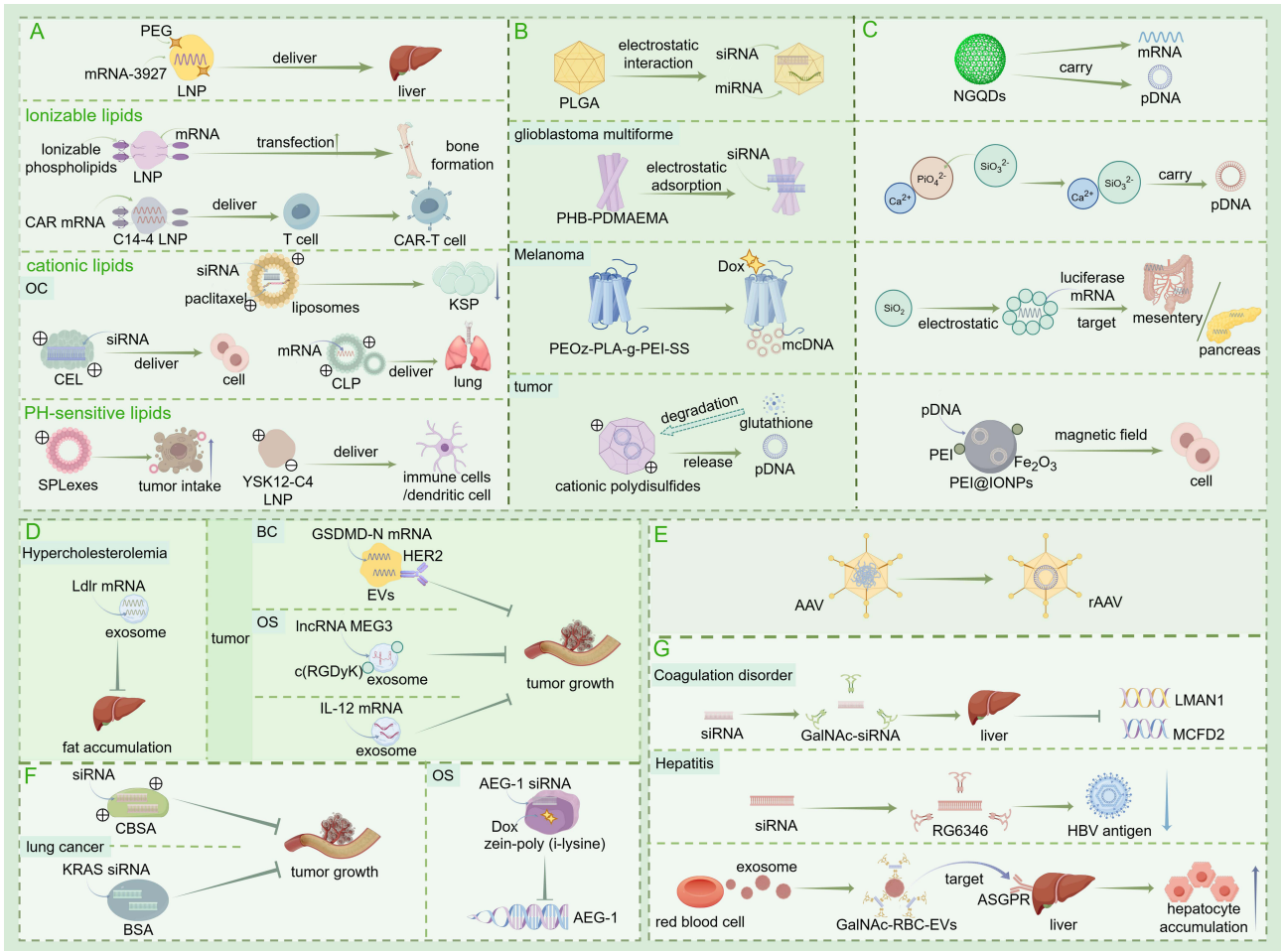


Figure 3 Delivery systems for nucleic acid therapeutics-including lipid-based, polymeric, inorganic, EV-based, viral, protein-based, and nucleic acid–conjugated platforms-enhance targeted delivery to specific cells and tissues, thereby improving therapeutic efficacy. (**A**) Lipid-based carrier strategies and associated therapeutic outcomes. (**B**) Polymeric nanocarriers for nucleic acid delivery. (**C**) Inorganic nanocarriers and their biological effects. (**D**) EV-mediated delivery systems and therapeutic functions. (**E**) Viral vector–based delivery platforms. (**F**) Protein nanocarrier strategies and associated therapeutic outcomes. (**G**) Nucleic acid–conjugated delivery systems and resulting therapeutic effects.

Notes: Upward and downward arrows indicate increases and decreases, respectively; T-shaped arrows denote inhibition or negative regulation; other arrows represent associations or process flow.

Abbreviations: PEG, polyethylene glycol; mRNA, messenger RNA; LNP, lipid nanoparticles; CAR, chimeric antigen receptor; OC, ovarian cancer; CEL, cationic cholesterol lipid derivative; siRNA, small interfering RNA; CLP, cationic lipid pair; PLGA, poly(lactic-co-glycolic acid); PHB-PDMAEMA, Angiopep-2 modified block copolymer micelle; PEOz-PLA-g-PEI-SS, poly(2-ethyl-2-oxazoline)-poly(L-lactide) combined with bio-reducible polyethyleneimine; Dox, Doxorubicin; mcDNA, minicircle DNA; pDNA, plasmid DNA; NGQDs, nitrogen-doped graphene quantum dots; PEI, polyethyleneimine; BC, breast cancer; GSDMD-N, N-terminal fragment of gasdermin-D; HER2, human epidermal growthFactor receptor 2; EVs, extracellular vesicles; OS, osteosarcoma; IL, interleukin; CBSA, cationic bovine serum albumin; BSA, bovine serum albumin; AEG-1, astrocyte elevated gene-1; AAV, adeno-associated virus; rAAV, Recombinant AAV; LMAN1, lectin mannose binding 1; MCFD2, multiple coagulation factor deficiency 2; HBV, Hepatitis B Virus; GalNAc, N-acetylgalactosamine; RBC, red blood cell; ASGPR, asialoglycoprotein receptor.

showed that ionizable lipid C14-4 LNPs could efficiently encapsulate CAR mRNA for delivery to T cells, as evidenced by fluorescence transfection experiments.[149]

Cationic lipids, such as the quaternary ammonium lipids 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine and 1,2-dioleoyl-3-trimethylammonium-propane—carry permanent positive charges that facilitate strong electrostatic interactions with negatively charged cell membranes, thereby enhancing intracellular transfection efficiency.[150–152] Current studies indicate that LNP systems containing cationic lipids are capable of encapsulating and delivering gene cargos across a broad size range, suggesting substantial translational potential.[130] For instance, Lee et al utilized PEGylated cationic liposomes loaded with siRNA to effectively silence kinesin spindle protein in ovarian cancer cells, resulting in reduced tumor growth.[153] Zhu et al developed a novel cationic cholesterol lipid derivative for siRNA delivery using microfluidic assembly.[154] Zeng et al introduced a strategy employing liver-targeted ionizable lipids and their quaternary ammonium

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derivatives as a cationic lipid pair for mRNA delivery, achieving a delivery shift from liver to lung and enabling efficient transfection of lung endothelial and epithelial cells.[155]

PH-sensitive lipids, which respond to pH changes during cellular transport, become positively charged as pH decreases,[147] thereby facilitating efficient cytosolic nucleic acid delivery.[156–158] Lin et al developed the sterically polymer-based liposomal complexes were formed by cationic polymeric liposomes and pH-sensitive diblock copolymer, significantly enhancing drug uptake in specific tumors.[159] Sato et al designed pH-sensitive cationic lipid YSK12-C4 LNPs, which achieved effective gene transfer in difficult-to-transfect murine bone marrow-derived dendritic cells and human immune cells.[160]

Polymeric Nanocarriers

Polymeric nanocarriers represent a delivery technology that utilizes natural or synthetic polymer materials to transport drugs through mechanisms such as adsorption and covalent bonding.[161] These carriers encapsulate drugs within a polymeric core or embed them internally to deliver the therapeutic agents to diseased sites within the body.[162] Polymeric nanocarriers exhibit diverse chemical and physical properties, allowing for the customization of their chemical composition and molecular weight based on the type of nucleic acid drug being delivered, thereby reducing side effects and enhancing therapeutic efficacy.[161,163,164] These carriers typically contain cationic groups, which aid in encapsulating negatively charged nucleic acid drugs and facilitate binding to negatively charged glycoproteins on cell membranes.[165] Currently, polymeric nanocarriers utilizing the proton sponge effect are widely employed for delivering nucleic acid drugs such as miRNA and siRNA.[166] Moreover, the use of biodegradable polymer materials prevents long-term accumulation in the body, reducing potential toxicity and enhancing biocompatibility (Figure 3).[167]

Poly(lactic-co-glycolic acid) (PLGA) delivers RNA complexes into cells through electrostatic interactions and can be chemically modified to improve in vivo efficacy and tolerance.[168,169] PLGA-based delivery systems have been used for various nucleic acid drugs, including siRNA and miRNA.[170–172] For instance, an Angiopep-2 modified block copolymer micelle (PHB-PDMAEMA) adsorbs therapeutic siRNA through electrostatic interactions, targets glioblastoma cells, and successfully inhibits the malignant biological effects of tumors.[173] Gaspar et al synthesized a tri-block copolymer micelle based on poly(2-ethyl-2-oxazoline)-poly(L-lactide) combined with bio-reducible polyethylenimine(PEI). This micelle can release bound mcDNA in response to specific stimuli and has been employed for the co-delivery of supercoiled mcDNA vectors and Doxorubicin (Dox), demonstrating significant ant0itumor effects in a melanoma mouse model.[174] Yu et al designed and synthesized a star-shaped cationic polydisulfide with a β-cyclodextrin core via disulfide exchange polymerization. This carrier can release pDNA through glutathione degradation within tumor cells, achieving effective tumor therapy with low toxicity both in vitro and in vivo.[18]

Inorganic Nanocarriers

Inorganic nanocarriers are delivery systems composed of inorganic materials, characterized by uniform surface potential and adjustable shapes and sizes.[5] These carriers are favored by researchers due to their unique electrical and optical properties, good biocompatibility, and low cytotoxicity.[175] The delivery of drugs using inorganic NPs is typically categorized into two approaches: one involves loading nucleic acid drugs directly into the interior of the NPs, while the other involves fixing the drugs onto the surface of the NPs through adsorption.[176] Common inorganic nanomaterials include gold, silica, calcium, carbon, and magnetic materials such as iron oxide (Figure 3).[177,178]

Carbon quantum dots are a representative inorganic nanocarrier, usually less than 10 nm in size, with several advantages, including low toxicity, high yield, strong water solubility, low photobleaching, and ease of surface modification.[179] These quantum dots are often surface-modified with substances such as polyethyleneimine, ethylene-diamine, spermidine, and arginine, enabling them to effectively deliver pDNA into cells.[179] Additionally, researchers have synthesized nitrogen-doped graphene quantum dots for the delivery of mRNA and pDNA.[180]

In gene delivery, calcium ions play a crucial role, particularly in enhancing pDNA delivery efficiency.[181] The transfection efficiency of pDNA in vitro can be significantly improved by appropriately substituting phosphates with silicates in nanostructured calcium phosphate.[182] Researchers have developed mesoporous silica NPs that can bind luciferase mRNA via electrostatic interactions and, upon intraperitoneal injection into mice, specifically express in the

pancreas and mesentery, achieving efficient cellular uptake and endosomal escape.[183] Professor Wang's team used cationic PEI to modify iron oxide NPs, constructing a novel DNA/PEI@IONPs delivery system.[184] This system, aided by a magnetic field, efficiently delivers negatively charged pDNA into cells and successfully achieves gene silencing, showcasing the enormous potential of inorganic nanocarriers in precision medicine and gene therapy.[184]

Extracellular Vesicles (EVs)

EVs are membrane-bound particles secreted by both eukaryotic and prokaryotic cells, playing an essential role in intercellular communication.[185] Owing to their non-immunogenicity, biodegradability, and excellent biocompatibility, EVs are considered highly promising delivery platforms.[186] In mammalian systems, EVs are generally categorized into three subtypes according to their size: exosomes (30–150 nm), microvesicles (100–1000 nm), and apoptotic bodies (100 nm–5 μm).[187,188] Among these, exosomes are the most widely utilized for nucleic acid drug delivery and are particularly effective carriers capable of crossing the blood–brain barrier.[189] In addition, plant-derived exosome-like nanoparticles, despite their storage challenges and intrinsic heterogeneity, have attracted increasing attention due to their abundant natural sources, unique intrinsic properties (such as anti-inflammatory and antioxidant activities), and outstanding biocompatibility and stability, making them promising candidates for further development (Figure 3).[190]

Current studies indicate that engineering strategies can be used to functionalize exosomal membranes with specific ligands or adhesion molecules.[191] These functional moieties interact with receptors on target cell membranes or mediate cellular internalization, thereby enabling precise targeting and enhancing intracellular drug release.[192] This capability renders exosomes exceptional vectors for the delivery of intracellular nucleic acid therapeutics.[193] Extensive research has also demonstrated their therapeutic potential in various chronic inflammatory diseases, including rheumatoid arthritis (RA), OA, atherosclerosis (AS), and inflammatory bowel disease (IBD).[194]

In the domain of gene therapy, Li et al investigated the forced expression of low-density lipoprotein receptor (Ldlr) in donor liver cells, encapsulating mRNA encoding Ldlr into exosomes.[195] Following intravenous injection, these exosomes efficiently delivered mRNA to the liver, significantly reducing lipid deposition and restoring receptor expression, thereby presenting a novel therapeutic strategy for familial hypercholesterolemia.[195] Additionally, Huang et al successfully targeted osteosarcoma (OS) and significantly inhibited its growth by combining exosomes modified with c(RGDyK) and the anti-tumor lncRNA maternally expressed gene 3.[191] Zhao's team developed an innovative EV-based delivery system that directly targets BC cells through human epidermal growth factor receptor 2 antibodies on the vesicle surface, delivering mRNA encoding the pyroptosis-inducing N-terminal fragment of GSDMD to tumor cells, effectively inducing apoptosis and significantly inhibiting tumor growth.[196] Liu et al prepared IL-12 mRNA exosomes for inhalation delivery, demonstrating significant anti-tumor effects in murine models. Compared to traditional liposomes loaded with IL-12 mRNA, these exosomes exhibited superior targeting, higher tumor cell uptake, and reduced systemic toxicity.[45]

Viral Vectors

Viral vectors have evolved into highly efficient delivery systems with specific cell type tropism, representing the only system capable of actively delivering payloads into the cell nucleus.[197,198] Despite early research indicating that viral vectors could successfully deliver therapeutic genes, their positive impact in clinical applications has been limited, often accompanied by significant immunotoxicity.[198] This has driven the development and translation of safer recombinant viral vectors.[199] Currently, gene therapy products mediated by recombinant viral vectors have received approval from the FDA and EMA, with adeno-associated virus (AAV) vectors being the primary carriers (Figure 3).[200,201]

Recombinant AAV vectors retain the capsid components and structure of wild-type AAV, but all viral coding sequences are replaced with therapeutic genes, thereby reducing immunogenicity.[201,202] AAVs possess broad tropism, allowing for the specific targeting of various tissues.[203] By modifying the interactions between capsid proteins and receptors, their cell-specific tropism can be regulated, promoting cellular uptake of the drugs.[203] However, the limited packaging capacity for nucleic acid drugs in AAV vectors restricts their clinical application.[204,205] To address this issue, strategies to reduce the size of the nucleic acid drugs have been proposed and investigated.[206,207]

Protein-Based Carriers

Protein-based carriers have garnered extensive research interest due to their low toxicity, biodegradability, and ability to interact with specific drugs and solvents.[208] Various proteins such as gelatin, albumin, silk fibroin, elastin, and zein have been developed for nucleic acid delivery applications.[209] Among these, albumin stands out as an excellent drug delivery carrier due to its ease of modification, long half-life, and multifunctional therapeutic payload capacity.[210] For instance, Han et al successfully delivered siRNA to the lungs by surface modification of cationic bovine serum albumin (BSA), significantly inhibiting tumor growth.[211] Additionally, researchers utilized BSA encapsulation to specifically silence alleles of genes such as KRAS mutations, demonstrating high loading capacity and low nanotoxicity.[102] Zein, owing to its hydrophobic nature, has been utilized for sustained siRNA delivery.[212] Pang et al developed a zein-polycation (poly-L-lysine) non-viral delivery system that electrostatically complexes siRNA, achieving pH-controlled release in lysosomal acidic microenvironments.[212] This system effectively targeted astrocyte elevated gene-1, facilitating gene silencing and co-treatment with Dox, thereby significantly suppressing OS progression (Figure 3).[212]

Nucleic Acid-Conjugated Delivery Systems

Nucleic acid-conjugated delivery systems directly couple non-coding RNAs to delivery materials, creating single-component drug delivery systems with defined properties. In this realm, N-acetylgalactosamine (GalNAc) conjugation technology has shown significant potential, particularly for liver-targeted delivery of oligonucleotides.[213,214] GalNAc-RNA conjugates can be administered via intravenous or subcutaneous injection.[214] Asialoglycoprotein receptor (ASGPR), an efficient endocytic receptor, expresses in high density on hepatocytes, making it an ideal receptor for liver-targeted nucleic acid drug delivery.[215–217] Currently, trivalent GalNAc serves as a high-affinity ligand for ASGPR.[218] Benefiting from the high density and rapid circulation of ASGPR, GalNAc-siRNA conjugates achieve efficient intracellular uptake.[215] ASGPR and clathrin-mediated endocytosis effectively promote GalNAc transport from the cell surface to the cytosol.[219] In ASOs and siRNA drugs, GalNAc conjugation technology has proven to be the most effective oligonucleotide delivery system, albeit primarily targeting liver cells.[220–222]

GalNAc conjugation technology offers significant advantages: it provides highly tissue-specific targeting to the liver.[218] This conjugate can be administered via subcutaneous injection, avoiding potential renal filtration issues associated with direct intravenous injection.[214] Leveraging inherent cellular uptake pathways, this technology demonstrates higher cellular entry efficiency.[215] Compared to nano-delivery systems, GalNAc technology offers higher safety profiles.[221] Ma et al designed an siRNA molecule conjugated with three GalNAc residues, which successfully achieved targeted hepatic delivery and suppressed the expression of mutant lectin mannose binding 1 and multiple coagulation factor deficiency 2 genes. This approach effectively treated the combined deficiency of coagulation factors V and VIII caused by these pathogenic variants.[223] In addition, RG6346, a double-stranded RNA interference agent conjugated with GalNAc and designed to target the S region of the hepatitis B virus (HBV) genome, markedly reduced intrahepatic HBV antigen levels following subcutaneous administration and is currently undergoing Phase I clinical evaluation.[224] Huang et al extracted red blood cell–derived EVs and modified them with triantennary GalNAc ligands, creating a novel delivery platform. This engineered system delivered multiple nucleic acid therapeutics to hepatocytes and demonstrated significantly higher hepatocellular accumulation compared with unmodified EVs through ASGPR-mediated uptake. The enhanced delivery efficiency translated into improved therapeutic outcomes across relevant disease models.[225]

Design, Mechanisms, and Clinical Applications of Nucleic Acid Drug Delivery Systems

Principles for Designing Nucleic Acid Drug Delivery Vehicles

The design of nucleic acid delivery vehicles has gradually shifted from the traditional concept of "whether delivery is feasible" to "how to deliver nucleic acids with precision, safety, and efficiency".[226] Recent advances emphasize overcoming physiological barriers and prolonging in vivo circulation time by modulating carrier characteristics such as particle size, chemical composition, and surface charge.[227,228] Surface modification with targeting moieties-including antibodies, peptides, or aptamers-can further enhance delivery specificity to particular cell types or tissues.[229] For microenvironment-responsive designs, such as in tumors, carriers must account for environmental factors including

acidic potential of hydrogen or elevated reactive oxygen species and employ strategies tailored to specific pathological niches.[230]

As summarized previously, different delivery platforms show distinct strengths and limitations. LNPs represent the most mature technology and have achieved successful clinical translation in several vaccines and therapeutics due to their high nucleic acid encapsulation efficiency and well-defined endosomal escape mechanisms. However, their repeated administration may elicit immunogenic responses, raising safety concerns. Polymeric nanocarriers offer highly tunable structures, but their intrinsic cationic properties often result in cytotoxicity and suboptimal delivery efficiency, hindering widespread clinical use. EVs exhibit excellent biocompatibility and an inherent ability to cross physiological barriers, such as the blood–brain barrier, yet their low nucleic acid loading efficiency and challenges in large-scale manufacturing limit current applications primarily to the research stage. GalNAc conjugates represent a paradigm of targeted delivery; with defined chemical structures, they enable highly specific hepatocyte targeting and have become the gold standard for liver-directed oligonucleotide therapeutics. Nevertheless, their application is largely confined to hepatic diseases.

In summary, the choice of delivery vehicle involves an inherent trade-off: LNPs serve as a highly efficient and versatile platform but require further safety optimization; GalNAc conjugates provide exceptional hepatocyte specificity but have limited applicability beyond the liver; EV-based delivery holds great promise yet demands technological refinement. To date, no delivery system is universally optimal-the selection should ultimately depend on the intended therapeutic context.

Intracellular Release and Activation Mechanisms of Nucleic Acid Drugs

Following cellular uptake of the delivery system, efficient endosomal escape is essential to ensure that nucleic acid therapeutics reach the cytoplasm intact. Most delivery carriers enter cells via endocytosis and are subject to lysosomal degradation if endosomal release is insufficient. To address this challenge, current strategies include the proton sponge effect, membrane fusion, membrane disruption, and viral-mimicking release mechanisms. Once released into the cytoplasm, nucleic acids must correctly interact with intracellular machinery to exert their therapeutic effects. For example, siRNA engages the RNA-induced silencing complex to mediate mRNA cleavage and achieve sustained gene silencing, while mRNA interacts with ribosomes for translation into functional therapeutic proteins. Successful activation of nucleic acid drugs thus requires maintaining their stability, activity, and specificity, as well as ensuring their accurate delivery to the cytoplasm or nucleus depending on the therapeutic modality.

Clinical Studies on Nucleic Acid Drug Delivery Systems

Although numerous nucleic acid delivery platforms have been developed, their clinical translation remains relatively limited (Table 1).

An LNP-encapsulated mRNA vaccine encoding HBV-related antigens is currently under evaluation in a Phase I clinical trial (NCT07077356). Preclinical studies have shown favorable tolerability and low toxicity, and further clinical assessment of its efficacy is ongoing. Additionally, intravenously administered LNPs loaded with tumor-associated mRNA preferentially target pulmonary antigen-presenting cells, inducing T-cell activation and antitumor immunity (NCT05660408). Another study (NCT06249048) is assessing the safety and antitumor activity of the LNP-formulated therapeutic STX-001 in patients with advanced solid tumors. A neutral liposomal formulation containing 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine is also being evaluated for siRNA delivery in patients with advanced or recurrent solid tumors (NCT01591356). Among viral vector systems, a Phase I trial (NCT06031727) is investigating the efficacy and safety of the AAV-based therapy HG202 for neovascular age-related macular degeneration. Another study (NCT06952842) is exploring rAAV-delivered ZVS203e for the treatment of RHO mutation–associated retinitis pigmentosa.

Clinical development of nucleic acid–conjugated therapeutics is likewise progressing. Pelacarsen has demonstrated favorable pharmacokinetics and safety in Phase I trials (NCT05026996).[231] Solbinsiran, a GalNAc-conjugated siRNA, has shown significant reductions in atherogenic lipoprotein levels (NCT05256654).[232] Vupanorsen, a GalNAc3-conjugated antisense oligonucleotide, demonstrated promising safety and efficacy in individuals with elevated triglycerides (NCT04916795).[233] Zodasiran, an RNA interference (RNAi) therapy targeting hepatic ANGPTL3 expression,

Table 1 Ongoing Clinical Trials Involving Nucleic Acid Drug Delivery Systems

Identifier	Phase	Patient Numbers	Delivery System	Drugs	Status	Estimated Study Completion Date	Objective/Outcome
NCT07077356	I	15	LNP	None	Recruiting	2027-12-31	To assess the vaccine's low toxicity, good tolerability, and potential.
NCT05660408	I, II	36	LNP	None	Recruiting	2035-10	To investigate safety and immunogenicity in pediatric patients with recurrent pHGG.
NCT06249048	I, II	108	LNP	STX-001	Recruiting	2028-11	To evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and antitumor efficacy of STX-001, alone or in combination, in advanced solid tumors.
NCT01591356	I	49	DOPC	EphA2 siRNA	Active, not recruiting	2027-04-30	To determine the safety, tolerability, maximum tolerated dose, and efficacy of EphA2 siRNA.
NCT06031727	I	12	AAV	HG202	Recruiting	2026-06-30	To evaluate the safety, tolerability, and efficacy of CRISPR-Cas13 RNA editing therapy (HG202) targeting VEGF-A in neovascular age-related macular degeneration (nAMD).
NCT06952842	I/II	18	rAAV	ZVS203e	Not yet recruiting	2045-06-18	To assess the safety, tolerability, and efficacy of subretinal ZVS203e injection.
NCT05256654	II	205	GalNAc	Solbinsiran	Completed	2024-05-23	Solbinsiran showed good efficacy and durability in reducing atherogenic lipoprotein levels in mixed dyslipidemia.
NCT04916795	I	18	GalNAc	Vupanorsen	Completed	2021-10-19	Vupanorsen demonstrated favorable pharmacokinetics, pharmacodynamics, and safety in subjects with elevated triglycerides, consistent with non-Chinese populations.
NCT04832971	IIb	204	GalNAc	Zodasiran	Completed	2024-09-25	Zodasiran led to significant triglyceride reduction at 24 weeks with good safety and efficacy.
NCT06586684	IV	40	GalNAc	Inclisiran	Not yet recruiting	2026-09-30	To evaluate the effects of Inclisiran on atherosclerosis and carotid plaque in patients.

Abbreviations: LNPs, lipid nanoparticles; PHGG, pediatric high-grade glioma; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine; siRNA, small interfering RNA; AAV, adeno-associated virus; rAAV, recombinant adeno-associated virus; GalNAc, N-acetylgalactosamine.

exhibited potent therapeutic effects in patients with mixed hyperlipidemia in a Phase IIb study (NCT04832971).[234] Inclisiran, a GalNAc-modified siRNA for treating atherosclerosis and carotid atherosclerotic plaque, has advanced to Phase IV clinical evaluation (NCT06586684), demonstrating strong clinical potential.

Current Research Status of Nucleic Acid Therapeutics in Metabolic Diseases

Nucleic acid therapeutics have emerged as promising agents in the study of metabolic disorders. Through modulation of critical gene expression, nucleic acid drugs such as siRNA and miRNA effectively target disease mechanisms. Recent advancements have centered on developing novel nucleic acid therapeutics and refining delivery systems to enhance bioavailability and specificity, thus opening new avenues for future therapeutic strategies despite ongoing challenges related to safety and stability (Table 2).

Table 2 Recent Research on Nucleic Acid Drugs for Three Metabolic Diseases

Pathology	Nucleic Acid Drugs	Delivery Vector	Administration Route	Mechanism of Action	Therapeutic Outcome
DM	pDNA	PST	Oral	pDNA encoding GPL1 gene targets ABST and NTCP, entering ECP	Single administration maintains normal glucose levels in DM mice/ monkeys for 7/14 days
DM	pDNA	CS-g-bPEI	Oral	Insulin(INS) DNA targets intestinal epithelial cells, some traverse the intestinal barrier, delivering to cells body-wide	Single administration sustainably reduces glucose levels in mice over several days
Type 1 DM	pDNA	L. lactis	Oral	Host cells internalize recombinant L. lactis carrying IL-4 and IL-10 pDNA for expression	Prevents hyperglycemia in mice, halts INS inflammation progression, and reduces pancreatic destruction
Type 2 DM	pDNA	Oleic acid-grafted-CS polymer modified with MAN or APH	Subcutaneous Injection	Mediated by GLUT-1 and prohibitin receptor, pDNA encoding shRNA targets ATMs or adipocytes, silencing TNFα and MCP-1	Significantly downregulates pro-inflammatory cytokines in DM mice, continuously improving fasting glucose, INS response, and glucose homeostasis
CLI and DM	pDNA	PIRES bicistronic plasmid	Intramuscular Injection	Injects pDNA encoding VEGF and HGF into limbs of CLI and DM patients, increasing serum growth factor levels	Controls blood glucose levels, improves ABI and angiogenesis, significantly reduces rest pain
DM Skin Wounds	mRNA and saRNA	DIM1T LNPs	Embedded in Wound	Carriers deliver mRNA encoding immune evasion protein E3 and saRNA encoding specific therapeutic proteins targeting ASCs' endocytic pathways, encapsulated in hydrogel and embedded in wounds	Increases ASCs' protein production, improves local immune environment, reduces inflammation, accelerates wound healing in DM
DPN	VM202	None	Intramuscular Injection	Injects VM202 into calf muscles, expressing two subtypes of HGF for two weeks, promoting angiogenesis and neuron survival	Well-tolerated, no significant adverse reactions, significantly relieves patient pain
Type 2 DM Fractures	pDNA	(PEI)-(pBMP-2 pFGF-2)	Implant	Local implant in lumbar spine and biceps femoris of DM rats with pDNA encoding BMP-2 and FGF-2	Significantly increases bone volume and surface area in DM rats
NAFLD/NASH	siRNA	LNP/GalNAc	Intravenous/ Subcutaneous Injection	Targets MCJ expression inhibition to enhance hepatocyte-mediated fatty acid β-oxidation, prevents ROS formation by reducing electron leak overload, increases ETC activity, accelerates lipid metabolism	Reduces lipid accumulation, degeneration, hepatocellular damage, fibrosis, lowers serum ALT levels, controls metabolic disorder
NAFLD	ASO and siRNA	GalNAc	Subcutaneous Injection	Post-transcriptional silencing of hepatic apoC3 expression at mRNA level	Upregulates LPL, reduces triglyceride levels, positively impacts NAFLD recovery, effective against acute pancreatitis and atherosclerosis

(*Continued*)

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Table 2 (Continued).

Pathology	Nucleic Acid Drugs	Delivery Vector	Administration Route	Mechanism of Action	Therapeutic Outcome
NAFLD	siRNA	GalNAc+SiO_2	Intravenous Injection	Optimized carriers enhance liver cell-specific targeting and internalization, silencing PCSK9 mRNA, significantly upregulating LDLR expression	Lowers serum LDL content, reduces lipid accumulation and liver damage, high safety profile
NAFLD	ASO	GalNAc	Subcutaneous Injection	Targets liver cells, silences PNPLA3, inhibits expression of PNPLA3 148M mutant protein, downregulates hepatic Mcp1	Improves liver fibrosis, reduces inflammatory response, increases collagen dissolution activity, reduces liver steatosis and NAFLD severity
NASH	siRNA	Galactose ligands	Subcutaneous Injection	Targets liver cells, binds cytoplasmic RISC, degrades HSD17β13 mRNA, reduces HSD17β13 protein production	Clinical studies show low drug adverse reactions, good tolerability, treatment lowers ALT levels, inhibits liver inflammation and fibrosis
NASH	miR-609	LNP	Intravenous Injection	Directly inhibits fibrogenesis in HSCs, lipid generation in hepatocytes, and inflammation in RHMs, induces proliferation and functional recovery of KCs	Inhibits all features of NASH phenotype, prevents diet-induced NASH in mice
NASH	siRNA	GalNAc	Subcutaneous Injection	Long-term targeting silencing of DGAT2 mRNA, inhibiting DGAT2 production	Reduces synthesis and accumulation of triglycerides, alleviates liver steatosis, but does not inhibit NASH inflammation and fibrosis, nor improve plasma cytokine levels
OP	miR-21-5p/ let-7b-5p	EVs	Intravenous Injection	miR-21-5p in ASC-EVs downregulates Acvr2α, inhibiting osteoclast differentiation, let-7b-5p significantly silences genes associated with osteoclast generation, synergizes with other factors in ASC-EVs	Significantly inhibits osteoclast generation and differentiation in OP mice, reduces bone loss, promotes bone healing
OP	siRNA	Cationic LNP	Intravenous Injection	Targets osteogenic lineage cells, inhibits Ckip-1 expression	Increases bone mass in OP mice, improves bone microstructure, enhances resistance to brittle fractures
OP	ASO	Asp^8-PU	Intravenous Injection	Targets bone resorption surface osteoclasts, inhibits miR214 expression, inhibits formation of osteoclasts in OP mice	Reduces osteoclast numbers, increases bone mass, improves bone microstructure
OP	siRNA	None (chemically modified)	Intravenous Injection	siRNA targeting miR-198-5p transfected into osteogenic cells in vitro	Reduces activity of osteogenic cells, induces apoptosis, delays OP progression
OP	lncRNA Nron	Asp^8-PU	Intravenous Injection	Overexpression of Nron in osteoclasts, upregulates Erα expression and enhances its stability	Inhibits bone resorption, significantly increases bone mass

(*Continued*)

Table 2 (Continued).

Pathology	Nucleic Acid Drugs	Delivery Vector	Administration Route	Mechanism of Action	Therapeutic Outcome
OP	ASO	OT-RBCEVs	Intravenous Injection	Specifically targets osteoclasts, inhibits miR-214 levels, reverses bone resorption	Significantly inhibits osteoclasts, activates osteoblasts, improves bone density in OP mice, increases trabecular bone numbers
OP	siRNA	Polymeric NP	Intraperitoneal Injection	Accumulates extensively on bone surfaces, interferes with Dcstamp mRNA expression, hinders cell fusion of preosteoclasts and bone resorption, promotes osteogenesis	Increases trabecular bone mass and microstructure in OP mice

Abbreviations: DM, Diabetes Mellitus; pDNA, plasmid DNA; PST, protamine sulfate-conjugated taurocholic acid construct supplemented with calcium phosphate; GPL-1, glucagon-like peptide-1; ASBT, apical sodium bile acid transporter; NTCP, sodium taurocholate cotransporting polypeptide; ECP, enterohepatic circulation pathway; CS-g-bPEI, chitosan grafted with branched structure polyethyleneimine; INS, insulin; L. lactis, Lactococcus lactis; IL, interleukin; CS, chitosan; MAN, α-D-mannopyranosylphenyl isothiocyanate; AHP, adipose homing peptide; GLUT-1, glucose transporter-1; TNFα, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein-1; CLI, Critical Limb Ischemia; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; ABI, Ankle-brachial index; DIM1T, isomannide-derived; saRNA, small activating RNA; mRNA, messenger RNA; LNPs, lipid nanoparticles; ASCs, adipose stem cells; DPN, diabetic peripheral neuropathy; (PEI)-(pBMP-2+pFGF-2), poly(lactic-co-glycolic acid) microparticles embedded in a fibrin gel surrounded by a collagen matrix that is permeated with polyethylenimine; BMP-2, bone morphogenetic protein-2; FGF-2, fibroblast growth factor-2; NAFLD, non-alcoholic fatty liver disease; NASH, Non-Alcoholic Steatohepatitis; siRNA, small interfering RNA; GalNAc, N-acetylgalactosamine; MCJ, Methylation-Controlled J protein; ROS, reactive oxygen species; ETC, electron transport chain; ALT, alanine aminotransferase; ASO, antisense oligonucleotides; apoC3, apolipoprotein C-III; LPL, lipoprotein lipase; PCSK9, proprotein convertase subtilisin kexin type 9; LDLR, low-density lipoprotein receptor; LDL, low-density lipoprotein; RISC, RNA-induced silencing complex; PNPLA3, patatin-like phospholipase domain-containing protein 3; Mcp1, monocyte chemoattractant protein 1; HSC, hepatic stellate cell; RHM, resident hepatic macrophage; KCs, kupffer cells; DGAT2, diacylglycerol O-acyltransferase 2; OP, osteoporosis; EVs, extracellular vesicles; Ckip-1, casein kinase-2 interacting protein-1 encoding gene; Asp^8-PU, polyurethane nanomicelles modified by the acidic peptide Asp^8; ERα, estrogen receptor α; OT-RBCEVs, osteoclasts-targeting red blood cell EVs; NPs, nanoparticles; Dcstamp, dendrocyte-expressed seven transmembrane protein.

Diabetes Mellitus (DM)

DM, a chronic metabolic disorder characterized by inadequate insulin(INS) secretion or INS resistance influenced by genetic and environmental factors,[235,236] has risen to become the third most prevalent non-communicable disease worldwide, following CVD and cancer.[237] Significant progress has been made in nucleic acid therapeutic research within the realm of DM treatment.

Shahriar et al engineered a triple padlock oral gene delivery platform integrating pDNA encoding glucagon-like peptide-1 with protamine sulfate-conjugated taurocholic acid construct supplemented with calcium phosphate (PST), forming a multi-modal gene complex (pDNA/PST).[238] This construct exploits apical sodium bile acid transporter and sodium taurocholate cotransporting polypeptide (NTCP) for targeted enterohepatic circulation pathway, achieving rapid reduction of non-fasting blood glucose levels in diabetic mice within 24 hours and sustained normalization for up to 7 days.[238] Lin et al utilized chitosan (CS) grafted with branched structure PEI for oral administration of INS pDNA, leveraging intestinal epithelial cell endocytosis for systemic cellular transfection and subsequent glucose uptake in muscle cells, thereby maintaining prolonged glycemic control in diabetic mice.[239] Preisser et al demonstrated that oral administration of Lactococcus lactis carrying pDNA encoding IL-4 and IL-10 lowers the incidence of DM in mice, mitigates hyperglycemia, and reduces pancreatic damage.[240] Sharma et al administered oleic acid-grafted-CS polymer modified with α-D-mannopyranosylphenyl isothiocyanate or adipose homing peptide subcutaneously to deliver four pDNA encoding short hairpin RNA (shRNA) targeting tumor necrosis factor α (TNFα) or monocyte chemoattractant protein-1, thereby selectively modulating adipose tissue inflammation and enhancing glucose homeostasis with pronounced anti-inflammatory and therapeutic effects.[241]

Furthermore, nucleic acid therapeutics exhibit potential in managing DM complications.[242] Yousefabad et al engineered adipose tissue-derived mesenchymal stem cells (MSCs) with recombinant lentiviral vectors expressing miR-126, transplanted these cells via muscle injection into critical limb ischemia diabetic rats, significantly ameliorating limb ischemia and necrosis, thereby restoring limb function.[243] Barć et al employed a dual-gene therapy approach by injecting plasmids encoding VEGF and HGF directly into the limbs of diabetic mice, promoting vascular and endothelial

regeneration with favorable tolerance and minimal complications.[244] Xue et al utilized isomannide-derived LNP to deliver mRNA encoding immune evasion protein E3 and saRNA encoding specific therapeutic proteins, targeting ASCs to induce sustained secretion of therapeutic proteins, thereby significantly improving wound healing in diabetic mice.[245] VM202 (Engensis), a non-viral pDNA product for treating painful diabetic peripheral neuropathy (DPN), has progressed to Phase III clinical trials by expressing HGF via muscle injection in the calves, effectively alleviating DPN-associated pain.[246] Khorsand et al demonstrated enhanced bone regeneration and accelerated fracture healing in a chronic diabetic animal model through combined delivery of pDNA encoding bone morphogenetic protein-2 and fibroblast growth factor-2 via PLGA microparticles embedded in a fibrin gel surrounded by a collagen matrix that is permeated with PEI implanting.[247]

Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD represents a predominant metabolic disorder characterized by hepatic lipid accumulation due to dysregulated lipid metabolism and excessive fatty acid transport within adipose tissue.[248–253] Recent years have witnessed substantial progress in nucleic acid therapeutics, particularly leveraging GalNAc conjugation delivery systems, for treating NAFLD. For instance, siRNA targeting the Methylation-Controlled J protein enhances hepatic cell-mediated fatty acid β-oxidation, significantly reducing lipid accumulation and mitigating hepatocellular injury and fibrosis.[254] Moreover, nucleic acid drugs targeting apolipoprotein C-III upregulate lipoprotein lipase, effectively lowering triglyceride levels, now advancing into clinical trial phases.[255] Degradable silica NPs conjugated with GalNAc demonstrate efficient and safe delivery of proprotein convertase subtilisin kexin type 9 gene-targeting siRNA to liver cells, substantially reducing hepatic damage.[256] Linden et al demonstrate that GalNAc-conjugated ASO-mediated silencing of patatin-like phospholipase domain-containing protein 3 effectively mitigates hepatic steatosis and improves liver fibrosis.[257]

NASH, an advanced form of NAFLD and a common cause of chronic liver disease,[258,259] has also seen significant therapeutic advancements. Mai et al developed ARO-HSD targeting the HSD17β13 gene, significantly lowering liver injury biomarkers through reduced hepatic HSD17β13 mRNA levels, demonstrating good safety and tolerability in clinical settings.[260] Additionally, studies utilizing miR-690 mimetics encapsulated in artificial liposomes effectively reduce liver fibrosis, steatosis, and inflammation in NASH mice.[121] GalNAc-conjugated siRNA targeting diacylglycerol O-acyltransferase 2 also shows promise in NASH therapy, inhibiting key triglyceride synthesis enzymes to effectively prevent and reverse hepatic lipid accumulation, thereby improving hepatic steatosis phenotype.[261] These studies not only underscore the efficacy of nucleic acid therapeutics in treating NAFLD but also provide a critical scientific foundation for further clinical applications.

Osteoporosis (OP)

OP is a metabolic bone disease triggered by multiple risk factors, characterized by reduced bone mass and microstructural changes, leading to decreased bone strength and increased fracture risk.[262,263] Recent studies indicate that EVs derived from ASCs, administered via intravenous injection, significantly attenuate bone loss in OP mice.[264] They suppress macrophage differentiation into osteoclasts and promote the migration of bone marrow-derived MSCs.[264] Notably, miR-21-5p in ASC-EVs downregulates Acvr2a to inhibit osteoclast differentiation, while let-7b-5p notably reduces gene expression associated with osteoclastogenesis.[264]

SiRNA targeting the negative regulator of bone formation, casein kinase-2 interacting protein-1 encoding gene (Ckip-1), has proven effective.[265] Gao et al demonstrated enhanced delivery of Ckip-1 siRNA to osteogenic lineage cells via intravenous injection of cationic LNPs systems, improving bone mass and microstructure in ovariectomy-induced OP mice and enhancing resistance to fragility fractures.[265] Cai et al successfully delivered anti-miR214 using polyurethane nanomicelles modified by the acidic peptide Asp8 (Asp8-PU) via intravenous injection to the bone resorption area, significantly inhibiting osteoclast formation and improving bone microstructure and mass in ovariectomized OP mice.[266] Jin et al identified the functional motif Nron of lncRNA as a key inhibitor of bone resorption. Intravenous injection of Asp8-PU bone-targeted delivery system significantly increased bone mass in Nron-treated mice compared to controls, confirming Nron's inhibitory effect on bone resorption.[124] Furthermore, intravenous injection of osteoclasts-targeting red blood cell EVs loaded with anti-miRNA-214 oligonucleotides significantly suppressed osteoclast activity, enhanced

osteoblast activity, and improved bone density in OP mice.[267] Zhang et al designed core-shell NPs composed of cationic and responsive core for controlling load and release of siRNA targeting dendrocyte-expressed seven transmembrane protein, enhancing circulation and bone-targeted delivery, inhibiting osteoclast fusion and bone resorption, and promoting osteogenesis.[268]

Current Research on Nucleic Acid Therapeutics in Inflammatory Diseases

Inflammatory Bowel Disease (IBD)

IBD represents a complex multifactorial condition characterized by chronic gastrointestinal inflammation, notably encompassing Crohn's disease (CD) and ulcerative colitis.[269–272] While conventional non-nucleic acid therapies are widely employed for IBD management, their efficacy is often limited in certain patients and may pose risks such as infections and cancer.[272–275] Consequently, nucleic acid therapeutics, distinguished by their enhanced safety and specificity profiles, have emerged as a promising frontier in IBD treatment (Table 3).

Studies reveal that orally administered ASO, encapsulated within CS hydrogels, demonstrate effective uptake by intestinal epithelial cells and macrophages, thereby significantly attenuating various inflammatory biomarkers including TNF-α.[276] Innovatively, Xiao et al developed galactosylated polymeric NPs loaded with anti-TNF-α siRNA and pro-regenerative IL-22, embedded in a hydrogel (CS/alginate), achieving targeted delivery to inflamed colonic regions.[277] This approach effectively suppresses TNFα expression and promotes mucosal healing.[277,278] Additionally, antibodies to β(7) integrintargeted stabilized NPs loaded with cyclin D1 (CyD1) siRNA silence specific leukocyte subsets involved in gut inflammation, reversing colitis and notably reducing TNF-α and IL-12 expression levels.[279,280] Ginger-derived LNPs systems, delivering CD98 siRNA orally, selectively downregulate CD98 expression in colonic tissues, underscoring their potential anti-inflammatory effects.[281] Moreover, Kriegel et al's multicompartmental biodegradable polymer-based NPs-in-microsphere oral system that combined with siRNA, concurrently silences TNF-α and CyD1 via dual-gene knockdown, thereby significantly mitigating colonic inflammation.[282]

In CD models, upregulated intercellular adhesion molecule 1 expression on inflamed mucosa is effectively inhibited via intravenous infusion of antisense phosphorothioate oligodeoxynucleotide ISIS 2302.[283] Elevated Smad7 levels, contributing to defective TGF-β1 signaling, represent a hallmark of IBD pathogenesis.[284] Oral administration of Smad7 ASO[284–286] and NF-κB (p65) antisense phosphorothioate oligodeoxynucleotides[287] demonstrate promising therapeutic potential in murine models. Furthermore, IL-18 overexpression in CD models is effectively attenuated through an E1/E3-deleted adenovirus expressing IL-18 antisense mRNA, leading to significant reductions in interferon-γ production within colonic mucosa.[288]

Rheumatoid Arthritis (RA)

RA is a debilitating autoimmune disorder characterized by persistent musculoskeletal pain, morning stiffness, and inflammatory synovitis affecting joints and adjacent tissues, often leading to erosive joint damage if left untreated.[289–294] Although conventional therapies are utility, but they are associated with significant adverse effects and therapeutic limitations.[295,296] Consequently, nucleic acid therapeutics present an appealing avenue due to their potential for enhanced safety and efficacy (Table 3).

In recent studies, Wang et al developed polymer hybrid micelle systems that effectively co-deliver dexamethasone (Dex) and NF-κB p65 siRNA, promoting polarization of M1 macrophages to an anti-inflammatory M2 phenotype in arthritis models, thereby mitigating inflammation.[297] Duan et al engineered hybrid-NPs system comprised of calcium phosphate/liposome, targeted via folate conjugation to activate macrophages, facilitating co-delivery of NF-κB siRNA and methotrexate to attenuate pro-inflammatory cytokine expression.[298] Similarly, Feng et al identified siRNA targeting endoplasmic reticulum to nucleus signaling 1, which, when delivered via cationic polymers, interfere with the function of inositol 1,4,5-trisphosphate receptor 1/3, regulate intracellular calcium flux, inhibit cytokine production, and induce M2 polarization of macrophage, showcasing therapeutic efficacy in arthritis models.[299] Zheng et al utilized human serum albumin for co-delivery of IL-10 pDNA and Dex sodium phosphate, effectively suppressing pro-inflammatory cytokine

Table 3 Recent Research on Nucleic Acid Drugs for Three Inflammatory Diseases

Pathology	Nucleic Acid Drugs	Delivery Vector	Administration Route	Mechanism of Action	Therapeutic Outcome
IBD	ASO	Chitosan hydrogels	Oral	Targets intestinal cells and macrophages, reduces myeloperoxidase and malondialdehyde, inhibits TNF-α production	Reduces inflammatory response, therapeutic effect in colitis mice
UC	siRNA	Galactosylated polymeric NPs	Oral	Targets inflamed colon, co-delivers anti-TNF-α siRNA with IL-22 to macrophages, downregulates TNF-α expression	Downregulates pro-inflammatory factors and significantly promotes mucosal healing
IBD	siRNA	β(7) I-tsNPs	Intravenous Injection	Inhibits leukocyte proliferation and Th1 cytokine expression, silences CyD1	Significantly reduces TNF-α and IL-12 expression, reverses colitis in mice
UC	siRNA	Ginger-derived LNPs	Oral	Delivers siRNA targeting CD98 in intestinal cells, inhibits CD98 production	Reduces colitis and colitis-associated inflammation
IBD	siRNA	NiMOS	Oral	Co-delivers siRNA for TNF-α and CyD1, dual gene silencing	Lowers TNF-α and CyD1 levels in colon, inhibits pro-inflammatory cytokine expression
CD	ISIS 2302	None (chemically modified)	Oral	Knocks down Smad7, restores TGF-β activity, inhibits production of inflammatory factors	Limits development of colitis and fibrosis
IBD	ASO	None	Colonic delivery	Reduces NF-κB p65 expression, significantly lowers inflammatory cytokine concentration	Early administration effectively inhibits inflammation in murine rectum
CD	ASO	AAV	Colonic injection	Inhibits IL-18 mRNA expression, downregulates IL-18 expression in colon cells and macrophages	Significantly inhibits colonic inflammation, locally suppresses mucosal IFN-γ production
RA	siRNA	Polymer hybrid micelle	Subcutaneous Injection	Co-delivers siRNA inhibiting NF-κB p65 and Dex, blocks activation of NF-κB p65, drives macrophage phenotype from pro-inflammatory to anti-inflammatory	Alleviates arthritis inflammation without damaging liver or kidney function
RA	siRNA	Hybrid nanoparticles	Intravenous Injection	Folate-conjugated, co-delivers NF-κB siRNA and MTX, targets macrophages, blocks NF-κB signaling pathway, reduces pro-inflammatory factors	Avoids adverse reactions of MTX, inhibits inflammation progression
RA	siRNA	Cationic polymers	Intravenous Injection	Targets to inhibit ERN1, IRE1α gene expression, inhibits inflammatory cytokine production, regulates intracellular calcium concentration in macrophages, adjusts immune homeostasis	Protects joints from inflammatory damage
RA	pDNA	HAS	Tail injection	Co-delivers IL-10 pDNA and DSP, targets to drive macrophages in synovial tissue from pro-inflammatory to anti-inflammatory phenotype	Lowers serum inflammatory cytokine levels, alleviates joint swelling and bone erosion, prevents bone damage, no significant drug toxicity

(*Continued*)

Table 3 (Continued).

Pathology	Nucleic Acid Drugs	Delivery Vector	Administration Route	Mechanism of Action	Therapeutic Outcome
RA	pDNA	M2-type exosomes	Tail injection	Co-delivers IL-10 pDNA and BSP, drives macrophages from pro-inflammatory to anti-inflammatory phenotype	Alleviates arthritis inflammation, high anti-inflammatory activity
RA	pDNA	UTMD	Muscle injection	Transfects pDNA encoding TNF-α receptor into inflamed joint muscles and synovial cells in rats, increases its expression, lowers TNF-α concentration	Significantly improves joint inflammation, reduces TNF-α and other inflammatory factors in synovial tissue and peripheral blood
RA	siRNA	Liposomes	Intravenous Injection	Contains siRNA in specialized LNP accumulates in inflamed joints in vivo, inhibits Pde3b expression, increases cAMP content, enhances expression of anti-inflammatory genes, promotes anti-inflammatory capability of macrophages	Reduces synovial hyperplasia, lowers inflammatory cell infiltration, alleviates joint inflammation, prevents damage to bones and cartilage, improves bone surface, increases number and thickness of trabeculae
RA	siRNA	FS14-NP	Intravenous Injection	Targets IL-1β siRNA rapidly accumulates in macrophages in arthritic joints, reduces IL-1β and pro-inflammatory factor production	Lowers expression of joint pro-inflammatory factors, inhibits progression of arthritis, reduces joint swelling, bone erosion, and cartilage damage
HBV	siRNA	Recombinant preS1-tP	Co-incubation (in vitro study)	Targets HBV NLS with siRNA specifically delivered to hepatocytes, induces HBV mRNA degradation, prevents core protein from entering nucleus, inhibits cccDNA formation	Inhibits HBV replication
HBV	siRNA	Recombinant preS1-tP	Co-incubation (in vitro study)	Delivers NLS siRNA to HepG2.2.15 cells, blocks rcDNA transport to nucleus	Significantly inhibits HBV replication, reduces HBV DNA and cccDNA levels
HBV	pDNA	None	Intraperitoneal injection/Splenic injection/ Intravenous injection	Administers MSCs and shRNA pDNA alone or in combination into mice, shRNA silences HBV gene expression via RNAi	Inhibits HBV expression in serum and liver tissue, significantly improves liver damage, combined injection shows reduced effect
HBV	ARC-520	ARC-EX1	Intravenous Injection	Targeted delivery to hepatocytes and entry into cytoplasm, degrades and blocks all cccDNA-derived virus transcription and translation	Reduces HBV antigen and DNA content, can lead to functional cure by restoring immune system

Abbreviations: IBD, inflammatory bowel disease; ASO, antisense oligonucleotides; TNFα, tumor necrosis factor α; UC, ulcerative colitis; siRNA, small interfering RNA; β(7) I-tsNPs, antibodies to β(7) integrintargeted stabilized nanoparticles; CyD1, cyclin D1; IL, interleukin; LNPs, lipid nanoparticles; NiMOS, multicompartmental biodegradable polymer-based nanoparticles-in-microsphere oral system; CD, crohn's disease; TGF-β, transforming growth factor-β; mRNA, messenger RNA; AAV, adeno-associated virus; IFN-γ, interferon-γ; RA, rheumatoid arthritis; Dex, dexamethasone; MTX, methotrexate; ERN1, endoplasmic reticulum to nucleus signaling 1; pDNA, plasmid DNA; HAS, hyaluronic acid serum; DSP, dexamethasone sodium phosphate; BSP, betamethasone sodium phosphate; UTMD, ultrasound targeted microbubble destruction; Pde3b, phosphodiesterase 3B; cAMP, cyclic adenosine monophosphate; HBV, Hepatitis B Virus; cccDNA, covalently closed circular DNA; tP, truncated protamine; NLS, nuclear localization sequence; rcDNA, relaxed circular DNA; MSCs, mesenchymal stem cells; shRNA, short hairpin RNA; RNAi, RNA interference.

production and enhancing anti-inflammatory cytokine expression, thereby alleviating arthritis symptoms.[300] Furthermore, M2-type exosomes co-delivering IL-10 pDNA and betamethasone sodium phosphate demonstrated notable therapeutic benefits.[301] Wang et al use ultrasound targeted microbubble destruction successfully transfected TNF-α receptor (TNFR) pDNA, ensuring sustained TNFR gene expression in rats and effectively alleviating RA symptoms.[302] Moreover,

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liposomes-loaded phosphodiesterase 3B siRNA, administered via intravenous injection in RA models by Chen et al, significantly attenuated synovial hyperplasia, thereby mitigating bone and cartilage destruction.[303] Additionally, Song et al reported that lipidoid-polymer hybrid NP (FS14-NP) loaded with IL-1β siRNA, administered via intravenous injection, accumulated in arthritic joints and effectively suppressed disease progression while alleviating joint swelling.[304]

HBV

HBV infection remains a major cause of acute and chronic hepatitis, necessitating effective antiviral strategies.[305] Current therapies primarily rely on interferon and nucleos(t)ide analogues.[306,307] Despite advances in these treatment options, research into nucleic acid drugs continues in search of more effective treatment options. Nucleic acid drugs function by inhibiting HBV DNA polymerase activity and viral replication, or through RNAi to silence viral gene expression (Table 3).

Zeng et al introduced an innovative nuclear localization sequence (NLS) siRNA delivery approach utilizing recombinant preS1-truncated protamine, targeted to HBV via NTCP, effectively suppressing HBV replication and infection.[308] Moreover, studies demonstrate that solely inhibiting HBV replication may be inadequate to restore liver function.[309] For instance, MSCs combined with shRNA-expressing plasmids in mouse models significantly suppressed HBV expression and attenuated liver damage.[309] Addressing the challenge posed by covalently closed circular DNA (cccDNA), a critical intermediate in HBV replication resistant to RNAi, ARC-520 emerges as a pivotal therapeutic.[310] Comprising synthetic siRNAs conjugated with cholesterol and formulated with polymer-based carriers (ARC-EX1), ARC-520 effectively reduces cccDNA-derived viral transcripts, halting viral protein translation and diminishing viral antigen and HBV DNA levels.[310] Building on previous findings,[308] Zeng et al underscore the potential of NLS-targeted siRNA in further reducing cccDNA levels.[311]

In drug development, ARC-520 represents the first approved nucleic acid therapeutic for HBV and is currently available commercially.[310] Despite the approval of lamivudine, challenges persist regarding its susceptibility to viral resistance and off-target effects.[312] Subsequent approvals of entecavir, adefovir, and tenofovir disoproxil fumarate for treating chronic hepatitis B, characterized by low resistance rates and high antiviral efficacy, have become standard treatment options. Recently, tenofovir alafenamide has gained approval due to its superior pharmacokinetic profile and reduced renal and skeletal adverse effects, highlighting its potential for enhanced therapeutic efficacy.[313]

Discussion

Nucleic acid therapeutics, owing to their unique biological attributes, not only modulate gene expression and transcriptional processes but also directly influence protein synthesis, thereby achieving therapeutic or disease-ameliorating effects. Although nucleic acid–based approaches such as RNA interference and mRNA therapy are already being applied in disease treatment, other modalities-including saRNA and shRNA-remain relatively underexplored. These agents hold strong theoretical promise but face substantial barriers to clinical translation, such as biosafety concerns, the risk of drug resistance and toxicity, and challenges related to in vivo stability and delivery efficiency.

To address these limitations, deeper insights into disease pathogenesis and more accurate identification of disease-specific molecular targets are essential. Multifaceted modulation of disease pathways may open avenues for innovative therapeutic strategies. For example, the combined use of different nucleic acid drugs may enhance therapeutic efficacy while reducing adverse effects. Moreover, optimizing delivery systems to improve the stability and pharmacokinetics of nucleic acids-such as through controlled-release carriers that activate drug expression under specific physiological conditions- represents a critical research direction.

Despite continuous progress in delivery technologies, each system still presents inherent limitations. LNPs tend to have suboptimal loading capacity, are rapidly cleared by the liver, and are costly to manufacture, contributing to potential hepatotoxic risks.[314] EVs exhibit low production yields, high heterogeneity, and batch-to-batch variability; their biological functions may differ across EV subpopulations, and current technologies cannot reliably isolate uniform EV fractions.[315] Inorganic nanoparticles often exhibit excessive solubility. Viral vectors suffer from manufacturing complexity, limited packaging capacity, insufficient targeting specificity, high immunogenicity, and potential oncogenicity.[316]

Protein-based carriers require improved endosomal escape performance and greater biostability to enhance delivery efficiency, and the use of non-human homologous proteins may further increase immunogenicity.[317] Nucleic acid conjugates generally display low endosomal escape rates; although chemical modification improves stability and targeting, such modifications may inadvertently raise immunogenicity due to structural alterations.

Although nucleic acid therapeutics have gained significant attention, only a small proportion have advanced to clinical trials. To overcome existing barriers, future studies must focus on accurately defining pathogenic mechanisms, validating disease-specific targets, and designing therapeutic strategies that intervene across multiple disease nodes. Further efforts should be directed toward optimizing delivery systems by engineering intelligent materials and integrating the release mechanisms of carriers with the activation mechanisms of nucleic acids. Such synergistic strategies may enhance stability, intracellular responsiveness, and tissue specificity. Rigorous preclinical and clinical evaluation is necessary to maximize therapeutic benefit and reduce systemic toxicity. Tailoring nucleic acid combinations and release strategies to individual disease profiles and patient characteristics is likely to represent an important future trend. Additionally, integrating the advantages of different carriers into hybrid delivery platforms may help overcome multiple biological barriers simultaneously. Ensuring manufacturing scalability, reproducibility, and quality stability while reducing production costs remains a major challenge. Long-term safety evaluation-including toxicity, immunogenicity, and biodegradability-is indispensable for ensuring clinical safety. Detailed studies of pharmacokinetics, biodistribution, and clearance mechanisms will be essential for optimizing dosing regimens and achieving efficient in vivo nucleic acid delivery.

In disease treatment, nucleic acid therapeutics have gained substantial interest in oncology and metabolic disorders such as diabetes. For instance, T-cell therapies engineered to target INS peptides and major histocompatibility complex class II molecules demonstrate the potential of immunocellular approaches in diabetes. Inspired by the remarkable success of CAR-T therapies in cancer, nucleic acid-based methods may offer novel strategies for chronic disease management through the controlled activation of immune cells, though further mechanistic studies and clinical validation are needed. Overall, nucleic acid therapeutics not only drive innovation in traditional treatment paradigms but also offer new hope for addressing complex and refractory diseases.

Notably, artificial intelligence (AI) has begun to play an increasingly important role in the design of nucleic acid drugs and delivery systems.[318] AI models trained on databases containing structural and performance data of delivery vehicles can predict efficacy, toxicity, and organ-targeting properties while virtually screening millions of candidate molecules to identify optimal design leads.[319] In the future, AI-guided design is expected to enable the tailoring of delivery systems to the unique physiological characteristics of individual patients, thereby improving clinical success rates.

Conclusion

Nucleic acid therapeutics have emerged as a transformative strategy for treating metabolic and inflammatory diseases, demonstrating substantial potential for clinical translation. In metabolic disorders, these therapeutics can repair damaged tissues by targeting disease-specific genes, promoting the expression of growth factors, and modulating cellular functions to achieve durable therapeutic outcomes. In inflammatory diseases, nucleic acids such as miRNAs and siRNAs effectively suppress the excessive expression of inflammatory mediators, thereby alleviating chronic inflammation and autoimmune symptoms.

The successful application of nucleic acid therapeutics depends largely on the design of efficient delivery systems. Lipid nanoparticles, polymer-based carriers, and viral vectors each provide distinct benefits in enhancing nucleic acid stability, bioavailability, and targeted tissue distribution. However, challenges remain in achieving optimal safety, stability, and controlled release. Key research priorities include:

Standardization of in vivo comparative frameworks: Unified evaluation models are essential for reliably assessing safety and efficacy across different delivery systems.

Scalable production of EVs: Developing cost-effective, large-scale EV manufacturing technologies is necessary for increasing clinical feasibility.

Clinical evaluation of combination therapies: Integrating nucleic acid therapeutics with traditional treatments or emerging modalities may yield enhanced efficacy for complex diseases such as cancer and autoimmune disorders.

Long-term safety assessment and immune monitoring: Given the novelty of these therapies, comprehensive safety data-including immunogenicity and risks of immune tolerance or autoimmunity-must be systematically collected.

Despite remarkable progress, multiple barriers hinder the commercial translation of nucleic acid therapeutics. High manufacturing costs, driven by complex production processes, must be reduced through improved large-scale technologies. Additionally, long-term stability, safety, and minimization of immunogenicity must be ensured before nucleic acid therapeutics can achieve widespread clinical implementation.

In conclusion, nucleic acid therapeutics represent a promising frontier for the treatment of metabolic and inflammatory diseases. Overcoming the remaining challenges in delivery, safety, scalability, and regulatory translation will be key to unlocking their full clinical potential and integrating them into mainstream medical practice.

Abbreviations

NPs, nanoparticles; EVs, extracellular vesicles; mRNA, messenger RNA; ASO, antisense oligonucleotide; miRNA, microRNA; siRNA, small interfering RNA; saRNA, small activating RNA; pDNA, plasmid DNA; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; AD, Alzheimer's disease; CRC, colorectal cancer; mcDNA, minicircle DNA; LNPs, lipid nanoparticles; iPSC, induced pluripotent stem cell; ASCs, adipose stem cells; CAR, chimeric antigen receptor; PCC, propionyl-coenzyme A carboxylase; IL, interleukin; PCa, prostate cancer; CVD, cardiovascular disease; circRNA, circular RNA; BC, breast cancer; GSDMD, gasdermin D; TS1, Timothy syndrome type 1; SMA, spinal muscular atrophy; aptamers, nucleic acid aptamers; TGF-β1, transforming growth factor-β1; lncRNA, long non-coding RNA; OA, osteoarthritis; NASH, non-alcoholic steatohepatitis; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PEI, polyethyleneimine; Dox, Doxorubicin; RA, rheumatoid arthritis; AS, atherosclerosis; IBD, inflammatory bowel disease; Ldlr, low-density lipoprotein receptor; OS, osteosarcoma; AAV, adeno-associated virus; BSA, bovine serum albumin; GalNAc, N-acetylgalactosamine; ASGPR, asialoglycoprotein receptor; HBV, Hepatitis B Virus; NAFLD, non-alcoholic fatty liver disease; RNAi, RNA interference; DM, Diabetes Mellitus; INS, Insulin; PST, protamine sulfate-conjugated taurocholic acid construct supplemented with calcium phosphate; NTCP, sodium taurocholate cotransporting polypeptide; CS, chitosan; shRNA, short hairpin RNA; TNFα, tumor necrosis factor α; MSCs, mesenchymal stem cells; DPN, diabetic peripheral neuropathy; OP, osteoporosis; Ckip-1, casein kinase-2 interacting protein-1 encoding gene; Asp8-PU, polyurethane nanomicelles modified by the acidic peptide Asp8; CD, Crohn's disease; CyD1, cyclin D1; Dex, dexamethasone; TNFR, TNF-α receptor; NLS, nuclear localization sequence; cccDNA, covalently closed circular DNA; AI, artificial intelligence.

Consent for Publication

All authors have read the manuscript and agreed to submit the paper for publication.

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.

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