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Navigating the next frontier in biomedicine: breakthroughs and insights in nucleic acid therapeutics

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Nucleic acid therapeutics are rapidly emerging as a transformative drug paradigm, offering precise and programmable regulation of gene expression across a broad spectrum of diseases. This review summarizes recent advances in key platforms—including antisense oligonucleotides, siRNA, miRNA, mRNA, and aptamers—emphasizing their unique mechanisms of action and therapeutic potential. We systematically outline critical contributions of chemical modification and delivery engineering, including backbone and sugar modifications, site-specific design, *N*-acetylgalactosamine (GalNAc) conjugation, and lipid nanoparticles, which collectively enhance stability, target specificity, and clinical applicability. Finally, we discuss persistent challenges such as immune activation, large-scale manufacturing, and long-term safety, and provide perspectives on future directions involving CRISPR-based gene editing, synthetic biology, nanotechnology, smart delivery systems, and combination therapies, aiming to offer strategic insights for the development and clinical translation of nucleic acid drugs.

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1. Introduction

The central dogma of molecular biology, a cornerstone of modern biology, highlights the critical role of nucleic acids in carrying genetic information.[1] These fundamental biomolecules are ubiquitous across all living organisms and govern essential life processes, such as growth, heredity, and variation.[2,3] With the advancement in molecular biology, nucleic acid-based therapeutics has emerged as a promising strategy for targeting pathogenic genes or mRNA, opening new avenues for disease treatment.[4]

Nucleic acid therapeutics leverage the sequence specificity and regulatory capacity of nucleic acids to influence gene expression and translation, enabling precise intervention through recognition of endogenous nucleic acid sequences.[5] Since the 1950s, breakthroughs in this field have been repeatedly honored with Nobel Prizes, underscoring both their scientific significance and clinical value. The elucidation of the DNA double-helix structure by Watson and Crick in 1953 established the molecular foundation for rational drug design.[6] Subsequently, the identification of catalytic RNAs (ribozymes) revealed that nucleic acids could serve

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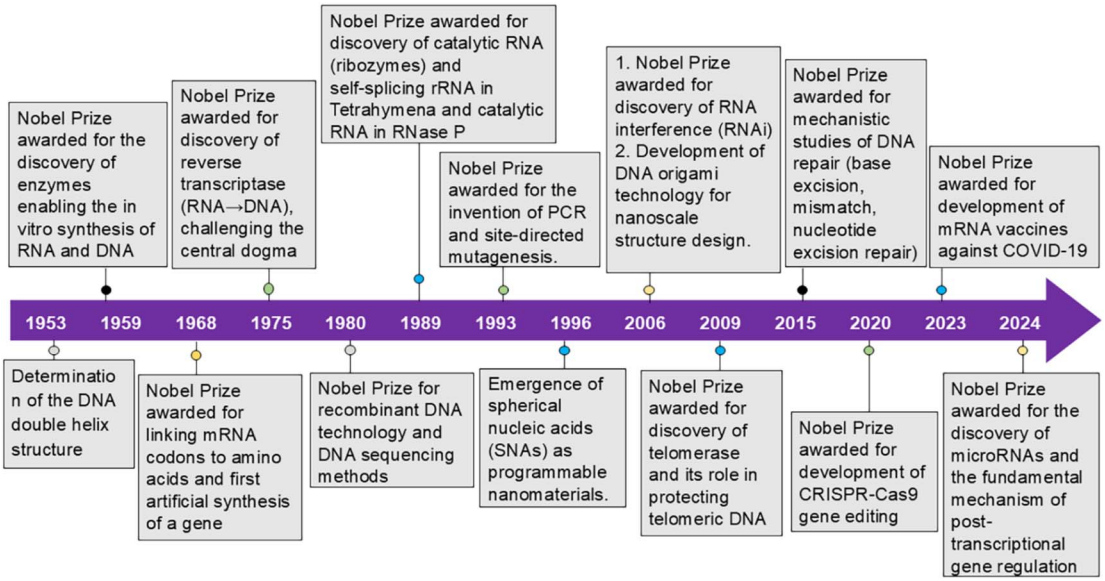

Fig. 1 Major breakthroughs and technological advances in nucleic acid science since the 1950s.

not only as carriers of genetic information but also as functional biomolecules, inspiring the development of riboswitches and, ultimately, nucleic acid aptamers as versatile molecular tools for gene regulation and therapeutic applications. Building on these advances, groundbreaking research in genetics led to the 2002 Nobel Prize, which deepened our understanding of genetic regulation in processes such as organogenesis and programmed cell death.[7] Parallel to these biological insights, the emergence of nucleic acid nanostructures introduced a new structural dimension to the field. In 1996, Chad A. Mirkin pioneered spherical nucleic acids (SNAs)—nucleic acid shells densely arranged on nanoparticle cores—establishing a new paradigm for programmable nanomaterials.[8] A decade later, in 2006, Paul W. K. Rothemund introduced DNA origami, demonstrating the precise folding of long DNA strands into two- and three-dimensional architectures.[9] These breakthroughs laid the foundation for the development of multifunctional DNA/RNA nanostructures with programmable shapes, spatial addressability, and biomedical applications, marking a transformative expansion of nucleic acid science beyond sequence information. In the 21st century, the discovery of RNA interference (2006) catalyzed the development of siRNA-based drugs, with the approval of Onpattro (patisiran) in 2018 as the first RNAi therapy, demonstrating the clinical feasibility of nucleic acid medicines.[10] The 2020 Nobel Prize awarded to CRISPR-Cas9, a gene-editing tool guided by RNA, further accelerated nucleic acid delivery and genome editing *in vivo*.[11] In 2023, the Nobel Prize recognized the role of nucleoside modifications in enhancing mRNA translation while evading immune recognition, laying the foundation for the success of mRNA vaccines.[12] In 2024, the Nobel Prize further acknowledged the therapeutic potential of microRNAs in post-transcriptional gene regulation, broadening the therapeutic landscape of nucleic acid-based therapies.[13]

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Collectively, these accolades not only affirm the extraordinary progress of nucleic acid science but also emphasize the transformative potential of nucleic acid therapeutics in the era of precision medicine (Fig. 1).[14,15]

Since the 1980s, the target-based approach to drug discovery has matured, driving the development of numerous innovative therapeutics.[16–18] Conventional small-molecule drugs and antibody-based biologics typically exert therapeutic effects by binding to target proteins such as enzymes, receptors, or ion channels.[19] Small molecules offer advantages including facile synthesis, oral bioavailability, favorable pharmacokinetic properties, and efficient membrane permeability.[20] However, their development is severely constrained by target "druggability." Among the ≈20 000 protein-coding genes in the human genome, only about 3000 are considered druggable, with just ≈700 yielding approved drugs.[21] Antibody therapeutics, by contrast, can target a wider array of proteins and can be engineered to improve their affinity and safety.[22,23] Nonetheless, their clinical application is limited by structural complexity, high manufacturing costs, and the need for parenteral administration.[24] Moreover, antibodies generally act only on extracellular or cell-surface proteins, significantly restricting their therapeutic scope.[25–27] In contrast, nucleic acid therapeutics offer unique advantages. They regulate gene expression through base-pair complementarity rather than direct protein binding, bypassing the limitations of protein "druggability". Furthermore, with appropriate delivery systems, nucleic acids can penetrate cells and act intracellularly, enabling broad regulation of intracellular, extracellular, and membrane-associated targets. Therapeutic nucleic acids can be designed rapidly based on known target gene sequences, with chemical modifications and delivery strategies developed independently. These capabilities position nucleic acid therapeutics as a transformative approach in precision medicine, offering novel solutions for both common and rear diseases, and overcoming the inherent limitations of traditional drug discovery.

2. Major classes of nucleic acid therapeutics and their recent clinical advances

2.1. Antisense oligonucleotides (ASOs)

2.1.1. Mechanisms of action of ASOs. Antisense oligonucleotides (ASOs) are a class of synthetic single-stranded DNA or RNA analogs, typically 15–30 nucleotides in length, that can precisely target RNA molecules through Watson–Crick base pairing. ASOs possess the potential to modulate RNA and protein expression, enabling inhibition, restoration, or modification of gene expression. Their molecular mechanisms mainly include steric hindrance, RNase H1-dependent degradation, splice reprogramming, and noncoding RNA regulation (Fig. 2A).[28,29] Steric hindrance refers to the binding of ASOs to critical functional regions of mRNA (such as the 5′ cap structure, the start codon, or ribosome-binding sites), resulting in a rigid complex that physically blocks ribosomal scanning. For example, mipomersen, used for the treatment of familial hypercholesterolemia, binds to the translation initiation site of ApoB-100 mRNA, thereby reducing LDL cholesterol levels by 36%.[30,31] RNase H1-mediated degradation represents a widely utilized strategy for gene regulation, as this enzyme is broadly expressed in both the nucleus and cytoplasm.[32] When the DNA segment of an ASO hybridizes with the target mRNA, RNase H1 is recruited to cleave the RNA strand. This process occurs in three steps: RNase H1 specifically recognizes the DNA–RNA heteroduplex *via* its hybrid-binding domain; subsequently

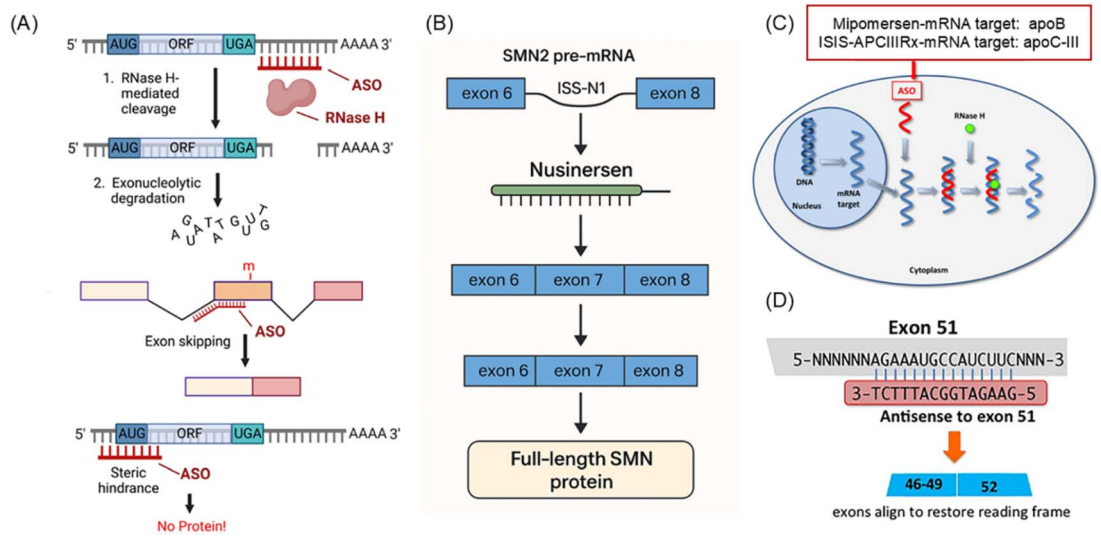


Fig. 2 Mechanisms of action of ASOs and representative drugs. (A) Pathways by which ASOs modulate pathological protein expression. (B) Nusinersen-mediated modification of SMN2 splicing. (C) Mechanism of action of mipomersen. (D) Eteplirsen-induced exon skipping to restore becker-type dystrophin. (A) Reproduced with permission from ref. 28. Copyright 2024, Springer. (B) Created by the authors using Microsoft PowerPoint. (C) Reproduced with permission from ref. 47. Copyright 2017 Elsevier. (D) Reproduced with permission from ref. 49. Copyright 2017, American Heart Association.

cleaves the phosphodiester bonds of the mRNA; and finally, the cleavage products are degraded by exonucleases. Such ASOs are commonly designed as Gapmers, consisting of a central region with no fewer than five consecutive DNA nucleotides flanked by wings of high-affinity modified nucleotides to enhance binding affinity and minimize off-target effects.[33] For instance, Emmrich *et al.* employed Gapmer-ASOs to successfully suppress the oncogenic splice isoform of p73. Aberrant p73 variants are frequently associated with poor prognosis and therapeutic resistance in various cancers; upon ASO treatment, the levels of oncogenic p73 transcripts and proteins in cancer cells were markedly reduced, thereby inhibiting apoptosis and attenuating tumor cell proliferation.[34]

Splice reprogramming refers to the modulation of pre-mRNA splicing within the nucleus, thereby altering the composition of mature mRNA. ASOs can bind to critical regions of pre-mRNA and, by blocking the interaction of inhibitory splicing factors or recruiting activators, promote the inclusion or skipping of specific exons to achieve selective splicing.[35] In 2013, Singh *et al.* first reported the therapeutic application of this mechanism: in cells derived from patients with spinal muscular atrophy (SMA), ASOs targeting the 3′-end of the intronic structure ISTL1 effectively corrected the exon-splicing defect of the SMN2 gene.[36] In addition, ASOs can also exert therapeutic effects by targeting long non-coding RNAs (lncRNAs). For example, in β-thalassemia, ASOs directed against an antisense lncRNA of the BCL11A gene were shown to increase fetal hemoglobin (HbF) expression by approximately 40%.[37] Furthermore, Yang *et al.* demonstrated that the lncRNA HIF1A-AS2 is regulated by the oncogene KRAS in lung cancer and promotes the proliferation of NSCLC. Inhibition of HIF1A-AS2 using ASOs markedly enhanced tumor sensitivity to both the MYC inhibitor 10058-F4 and cisplatin treatment.[38]

2.1.2. Clinical applications of ASOs. Since the approval of the first ASO drug, fomivirsen, by the FDA in 1998, ASO-based therapies have achieved remarkable progress. Fomivirsen, a phosphorothioate-modified ASO, was approved by the FDA in 1998 and subsequently by the EMA in 1999 as a second-line therapy for cytomegalovirus (CMV) retinitis in patients with acquired immunodeficiency syndrome (AIDS). As of December 2023, a total of ten ASO drugs have been approved by the FDA, covering a broad spectrum of therapeutic areas including metabolic/endocrine, neurological/muscular, cardiovascular, and infectious diseases (Table 1).[39] These drugs are administered through multiple routes, such as subcutaneous injection, intravenous infusion, intravitreal injection, and intrathecal injection. In the following section, several representative ASO drugs will be highlighted.

Nusinersen (Spinraza) is an ASO approved for the treatment of SMA. SMA is primarily caused by mutations in the survival motor neuron 1 (SMN1), leading to deficiency of the SMN protein.[40] Nusinersen is a splice-modulating ASO that specifically binds to the intronic splicing silencer N1 (ISS-N1) region of SMN2 pre-mRNA. SMN2 is a gene highly homologous to SMN1, but its transcripts typically undergo exon 7 skipping, resulting in truncated and nonfunctional proteins.[41] By blocking the binding of inhibitory splicing factors to ISS-N1, and potentially recruiting activators, nusinersen promotes the inclusion of exon 7 in SMN2 pre-mRNA, thereby significantly increasing the production of full-length, functional SMN protein (Fig. 2B).[42] The drug is delivered *via* intrathecal injection, enabling it to bypass the blood–brain barrier and reach the cerebrospinal fluid to target spinal motor neurons. Multiple pivotal clinical trials have demonstrated that nusinersen markedly improves motor function, survival, and respiratory capacity in SMA patients, with particularly pronounced efficacy in presymptomatic and early-onset infants.[43] As the first approved therapy for SMA, nusinersen has fundamentally altered the natural course of the disease.[44]

Mipomersen (Kynamro) is an antisense oligonucleotide that targets the Apolipoprotein B-100 (ApoB-100) mRNA and is approved for the treatment of homozygous familial hypercholesterolemia (HoFH). HoFH results from mutations in the ApoB-100 gene, leading to loss of low-density lipoprotein (LDL) receptor function. ApoB-100 is a structural protein required for the hepatic synthesis of very-low-density lipoprotein (VLDL), which is subsequently metabolized into LDL.[45] Mipomersen is a 2′-*O*-methoxyethyl (2′-MOE)-modified Gapmer ASO that promotes the degradation of ApoB-100 mRNA *via* RNase H1 activation.[46] Following subcutaneous administration, the drug inhibits hepatic ApoB-100 synthesis and reduces VLDL secretion, thereby significantly lowering plasma LDL cholesterol levels (Fig. 2C).[47] However, its use is associated with hepatotoxicity risks inhibition of VLDL secretion leads to triglyceride

Table 1 FDA-approved antisense oligonucleotide (ASO) drugs, their brand names, approval years, and indications (as of 2023)

Drug name	Brand names	Approval year	Indication
Fomivirsen	Vitravene	1998	CMV retinitis in AIDS patients
Mipomersen	Kynamro	2013	Homozygous familial hypercholesterolemia (HoFH)
Nusinersen	Spinraza	2016	Spinal muscular atrophy (SMA)
Eteplirsen	Exondys	2016	Duchenne muscular dystrophy (DMD), exon 51 skipping
Inotersen	Tegsedi	2018	Hereditary transthryetin-mediated amyloidosis (hATTR-PN)
Volanesorsen	Waylivra	2019	Familial chylomicronemia syndrome (FCS)
Golodirsen	Vyondys 53	2019	DMD, exon 53 skipping
Viltolarsen	Viltepso	2020	DMD, exon 53 skipping
Casimersen	Amondy 45	2021	DMD, exon 45 skipping
Tofersen	Qalsody	2023	SOD1 mutration-associated amytrophic lateral sclerosis (ALS)

accumulation in hepatocytes, resulting in elevated transaminase levels ≥3× the upper limit of normal (ULN) in approximately 10–15% of patients.

Eteplirsen is an antisense oligonucleotide developed for the treatment of Duchenne muscular dystrophy (DMD). DMD is caused by mutations in the DMD gene, resulting in loss of dystrophin protein, which disrupts muscle membrane stability and leads to progressive muscle degeneration.[48] Patients typically lose ambulation before the age of 12 and die from cardiorespiratory failure before age 20. Eteplirsen is an exon-skipping ASO designed for patients with mutations amenable to exon 51 skipping. These mutations induce a frameshift and premature stop codons, yielding nonfunctional ytruncated proteins. Eteplirsen belongs to the class of phosphorodiamidate morpholino oligomers (PMOs) and targets the splicing enhancer within exon 51 of DMD pre-mRNA. By binding to this regulatory region, it creates steric hindrance and blocks the recognition of exon 51 by the splicing factor U1 snRNP, thereby inducing exon 51 skipping. This allows exons 50 and 52 to be joined directly, restoring the reading frame and producing a shortened but partially functional Becker-type dystrophin protein (Fig. 2D).[49] Clinical trials demonstrated increased dystrophin expression in muscle biopsies from eteplirsen-treated patients, along with delayed decline in the six-minute walk test.[50] Notably, eteplirsen became the first drug in history to be conditionally approved for DMD based on a surrogate endpoint.

2.1.3. Impact of chemical modifications on ASOs. The intermediate molecular size of ASOs enables effective distribution to target tissues through multiple routes of administration. However, unmodified ASOs are rapidly degraded by serum nucleases *in vivo* and quickly eliminated from circulation *via* renal filtration. Consequently, chemical modifications are essential to enhance nuclease stability, target recognition, binding efficiency, and tissue distribution of ASOs, while also reducing their potential toxicity. Currently, the principal chemical modification strategies of ASOs are illustrated in Fig. 3A.[51]

2.1.3.1. Backbone modifications. Phosphorothioate (PS) modification involves replacing the non-bridging oxygen atom of the phosphate backbone with sulfur. PS linkages exist as two stereoisomers, whereas natural phosphodiester bonds are prochiral. PS-ASOs synthesized *via* conventional methods generally consist of mixtures of diastereomers, with certain stereoisomers displaying higher activity. This modification has become one of the most widely applied chemical strategies.[52] Phosphoramidate (PN) modification substitutes oxygen with nitrogen to form a P–N bond, while phosphorodiamidate borane (PB) modification introduces a tetrahedral structure *via* boron incorporation, which can increase the melting temperature (T_m) by approximately 8 °C; these modifications are currently in preclinical development. Such backbone modifications significantly enhance nuclease resistance and prolong the *in vivo* half-life of ASOs. Moreover, the negative charge facilitates binding to plasma proteins (*e.g.*, albumin), thereby improving pharmacokinetics, enhancing tissue distribution, and promoting cellular uptake.[53] Nevertheless, these modifications may reduce RNA-binding affinity and increase nonspecific protein interactions, potentially leading to adverse effects such as thrombocytopenia or nephrotoxicity. Phosphorodiamidate morpholino oligomers (PMOs) represent another backbone modification strategy, in which the ribose sugar is replaced by a morpholine ring and phosphorodiamidate linkages are introduced. This modification not only enhances nuclease resistance but also confers minimal immune activation potential.

2.1.3.2. Sugar modifications. Major sugar ring modifications include 2′-OMe, 2′-MOE, locked nucleic acid (LNA), and 2′-fluoro (2′-F). The 2′-OMe modification improves nuclease resistance, increases binding affinity (as reflected by elevated T_m values), and reduces immunogenicity. The 2′-MOE modification further increases hydrophobicity, resulting in superior nuclease resistance, higher binding affinity, and lower immunogenicity; however, the increased molecular weight may impair *in vivo* delivery efficiency. LNA modifications introduce a rigid methylene bridge between the 2′-O and 4′-C positions, markedly enhancing both binding affinity and nuclease stability.[54,55] LNA has been widely applied to enhance the potency of short ASOs; however, potential hepatotoxicity—particularly associated with TGC/TCC motifs—remains a safety concern. Yoshida *et al.*[56]

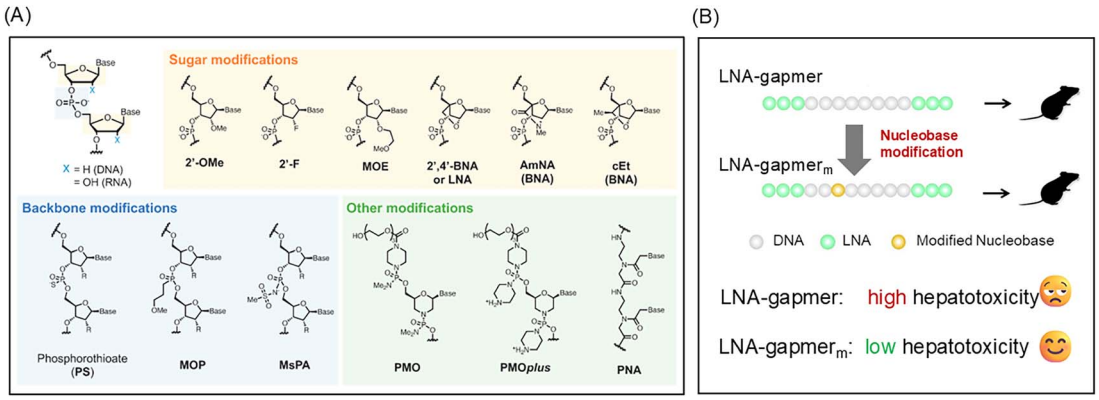


Fig. 3 Chemical modifications of ASOs. (A) Schematic representation of common chemical modifications in ASOs. (B) Nucleobase modification strategies to reduce hepatotoxicity of LNA-gapmers. (A) Reproduced from ref. 51. Licensed under a Creative Commons CC BY-NC 4.0 License. (B) Reproduced from ref. 56. Licensed under a Creative Commons CC BY-NC 4.0 License.

systematically screened 17 nucleobase derivatives and 4 novel modifications, identifying several that significantly reduced the hepatotoxicity of LNA-ASOs (Fig. 3B). The 2′-F modification also provides improvements in nuclease stability and binding affinity,[57] although it may partially inhibit the activation efficiency of RNase H.

The terminal nucleotides of ASOs can be functionalized *via* conjugation strategies with specific ligands (such as GalNAc, cholesterol, peptides, or antibody fragments), thereby enabling active targeting of cell surface receptors. This approach markedly enhances cellular uptake efficiency, reduces systemic dosing requirements, and minimizes off-target effects and toxicity. Among these, GalNAc-ASO conjugates efficiently target the asialoglycoprotein receptor (ASGPR) and have been extensively applied for liver-specific delivery.[58,59] In terms of structural design, Gapmers integrate multiple modification advantages: the central region, typically composed of 8–10 DNA or phosphorothioate-DNA nucleotides, is responsible for recruiting RNase H1, whereas the flanking wings are modified with 2′-OMe, 2′-MOE, or LNA nucleotides to enhance binding affinity and stability. Seth and colleagues reported a site-specific incorporation strategy in which 5′-methyl DNA nucleotide stereoisomers were introduced into the Gapmer region.[60] Their systematic evaluation demonstrated that placing such modifications at the third and fourth positions enhanced the therapeutic performance of PS-ASOs while modulating cytotoxicity, highlighting the clinical potential of this design. It should be noted, however, that ASO modifications must be rationally balanced: excessive modifications (*e.g.*, full phosphorothioation or an abundance of high-affinity substitutions) may lead to overly strong binding, off-target effects, or increased toxicity.

2.2. MicroRNA

2.2.1. Mechanisms of MicroRNA action. MicroRNAs (miRNAs) are a class of endogenous non-coding single-stranded RNAs, approximately 20–30 nucleotides in length, that act as key post-transcriptional regulators of gene expression. They are currently estimated to modulate the expression of more than 60% of protein-coding genes.[61] The biogenesis of miRNAs begins with the transcription of genomic DNA to produce primary miRNAs (pri-miRNAs) with characteristic stem-loop structures.[62,63] These pri-miRNAs are subsequently processed by the microprocessor complex, which consists of the RNase III endonuclease Drosha and two double-stranded RNA-binding domains of DGCR8, generating precursor miRNAs (pre-miRNAs) that retain the stem-loop structure (Fig. 4A).[62] Pre-miRNAs are then exported to the cytoplasm by Exportin-5 (Xpo5), where they are further cleaved by the RNase III enzyme Dicer into miRNA duplexes. Following duplex unwinding, one strand is selectively incorporated into the RNA-induced silencing complex (RISC), while the complementary strand is degraded. Ultimately, the miRNA-RISC complex scans and recognizes complementary mRNA sequences to mediate target mRNA silencing. Target recognition is largely mediated through the 5′ seed sequence (nucleotides 2–8) of miRNAs, which typically pairs imperfectly with the 3′ untranslated

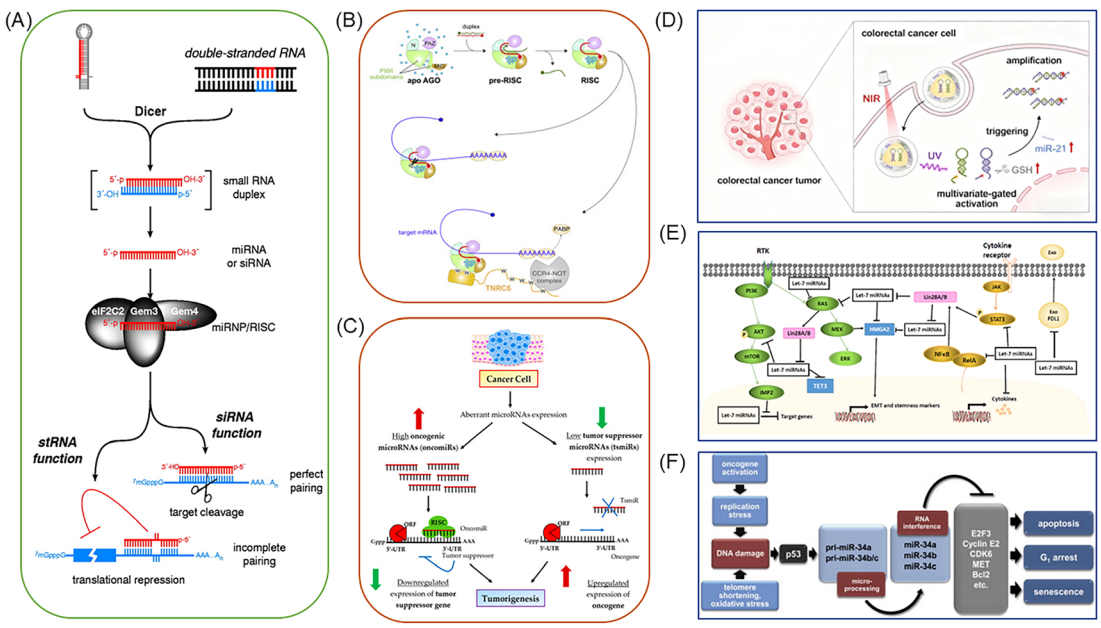


Fig. 4 Mechanisms of miRNA action and representative examples. (A) Schematic model of miRNA biogenesis and common pathways of mRNA regulation. (B) Tripartite model of RISC assembly. (C) Regulatory mechanisms of oncogenic and tumor-suppressive microRNAs in cancer. (D) Spatially selective microRNA imaging in human colorectal cancer tissues using a multivariate logic-gated signal amplification nanosensor. (E) Heatmap of let-7 family expression in benign breast tumors and invasive ductal carcinoma specimens. (F) miR-34 family as mediators of p53-dependent tumor suppression. (A) Reproduced with permission from ref. 62. Copyright 2002, American Association for the Advancement of Science. (B) Reproduced with permission from ref. 65. Copyright 2019, Cell Press. (C) Reproduced from ref. 66. Licensed under a Creative Commons CC BY-NC 4.0 License. (D) Reproduced with permission from ref. 78. Copyright 2025, American Chemical Society. (E) Reproduced from ref. 79. Licensed under a Creative Commons CC BY-NC 4.0 License. (F) Reproduced with permission from ref. 84. Copyright 2007, Elsevier.

regions (3′-UTRs) of target mRNAs.[64–66] When miRNA–mRNA complementarity is high, Ago2—the catalytic core of RISC—can directly cleave the target mRNA (Fig. 4B).[65] Even with partial complementarity, the miRNA–RISC complex can inhibit translation through multiple mechanisms, including blockade of ribosome assembly, interference with elongation, promotion of ribosome drop-off, or sequestration of target mRNAs into processing bodies (P-bodies) for storage or degradation.[67–70] Notably, this regulatory process is characterized by its multi-target nature: a single miRNA can regulate hundreds of genes. Moreover, the dynamic assembly of RISC mediated by AGO2 allows for dose-dependent fine-tuning of gene expression.

2.2.2. Clinical relevance of miRNAs. Dysregulation of miRNA expression is a critical hallmark and driving factor in the pathogenesis of numerous diseases.[71] For instance, oncogenic miRNAs (OncomiRs) are aberrantly upregulated in tumors, where they promote tumor initiation and progression by suppressing tumor suppressor genes and regulating pathways associated with cell cycle, apoptosis, invasion, and metastasis (Fig. 4C). miR-21, one of the most prevalent OncomiRs,[72] is significantly overexpressed in the majority of solid tumors[73,74] as well as in certain hematologic malignancies.[75,76] It targets multiple tumor suppressor genes simultaneously: inhibition of PTEN leads to hyperactivation of the PI3K/AKT pathway, thereby promoting proliferation and migration; suppression of PDCD4 enhances epithelial–mesenchymal transition (EMT) and metastatic potential; and downregulation of TIMP3 and RECK accelerates extracellular matrix (ECM) degradation. Because inhibition of miR-21 can concomitantly restore the function of several tumor suppressors, it has emerged as a highly promising anticancer target. Ma *et al.*[77] reported that miR-21 is overexpressed in a mouse model of acute pancreatitis.

In addition, Yuan and colleagues[78] developed a multivariate-gated catalytic hairpin assembly (CHA) nanosensor that enabled specific amplified imaging of miR-21 in colorectal cancer tissues (Fig. 4D) and further demonstrated that miR-21 contributes to colorectal tumorigenesis by suppressing the expression of the mismatch repair protein hMSH2. Conversely, tumor-suppressive miRNAs (TSmiRs) are frequently downregulated or lost in tumors, where their physiological role is to inhibit the expression of oncogenes. Thus, restoration of TSmiR expression is considered to have therapeutic potential. The let-7 family represents one of the earliest discovered TSmiRs, and reduced expression levels have been strongly associated with shorter postoperative survival in cancer patients. Forced expression of let-7 in both *in vitro* and *in vivo* models effectively suppressed tumor growth (Fig. 4E). Let-7 exerts its tumor-suppressive effects primarily by directly targeting multiple oncogenes, including RAS, MYC, and HMGA2.[79] Consequently, diminished expression of let-7 has been recognized as a prognostic biomarker for predicting survival outcomes in lung cancer patients.[80]

2.2.3. miRNA-Based therapeutics. As the roles of miRNAs in disease pathogenesis are increasingly elucidated, their therapeutic potential across diverse pathological processes has garnered substantial attention. Therapeutic development based on miRNAs has primarily focused on two strategies: miRNA inhibitors and miRNA mimics. miRNA inhibitors silence or block the function of oncogenic miRNAs,[81] and their design is conceptually similar to that of ASOs, requiring chemical modifications to enhance stability, binding affinity, delivery efficiency, and to reduce toxicity. A representative example is miravirsen, a 15-nucleotide oligonucleotide that targets miR-122 in hepatocytes.[82] miR-122 stabilizes hepatitis C virus (HCV) RNA by binding to the 5′-untranslated region, thereby acting as a critical host factor for HCV replication. Miravirsen sequesters miR-122 through complementary binding and thereby inhibits its function. In clinical trials, miravirsen demonstrated significant reductions in HCV viral load, with favorable efficacy and safety profiles.[83]

Conversely, miRNA mimics are designed to supplement or restore the function of tumor-suppressive miRNAs. To enhance stability and delivery efficiency, they are typically modified with 2′-OMe or PS chemistries. These double-stranded molecules are often encapsulated in lipid nanoparticles (LNPs) or other delivery vehicles, which protect their duplex structure, facilitate cellular uptake, and promote endosomal escape. A representative example is MRX34, a liposomal miR-34a mimic, which was the first miRNA-based therapeutic developed for cancer. miR-34a, the major member of the miR-34 family, is transcriptionally regulated by the tumor suppressor p53, which is frequently mutated or deleted in cancers. In most malignancies, miR-34a is downregulated (Fig. 4F).[84] MRX34 exhibited antitumor activity in phase I clinical trials across various solid tumors, reducing the expression of miR-34 target genes, oncogenes, and immune evasion-related genes. However, its development was ultimately discontinued due to severe immune-related adverse events.[85,86]

2.3. siRNA

2.3.1. Development and primary mechanisms of siRNA. Small interfering RNA (siRNA) originated from the discovery of RNA interference in 1998 and the subsequent demonstration in 2001 that 21–23 nt double-stranded siRNAs could efficiently and sequence-specifically silence genes in mammalian cells.[87,88] Subsequent molecular elucidation of the Dicer–Ago pathway, together with the systematic application of chemical modifications such as 2′-OMe/2′-F substitutions and PS end caps, markedly reduced nuclease degradation and innate immune activation (*e.g.*, TLR7/8), thereby laying the foundation for *in vivo* therapeutics.[89] Over the past decade, delivery paradigms have been clinically established: (i) trivalent GalNAc ligands, consisting of three GalNAc residues displayed on a branched scaffold, exhibit high affinity for ASGPR on hepatocytes and enable efficient receptor-mediated endocytosis (Fig. 5A), thereby making subcutaneous administration the mainstream route and reducing dosing frequency to quarterly or biannual regimens. (ii) Lipid nanoparticles (LNPs), taken up by hepatic sinusoidal endothelium and trafficked through the ApoE/LDLR pathway, enabled the approval of the first siRNA therapeutic in 2018 (Fig. 5B).[90] Benefiting from these two technological routes, siRNA indications have expanded from rare amyloidosis and metabolic genetic disorders to more common hepatic metabolic and cardiovascular risk factor-related diseases, establishing an

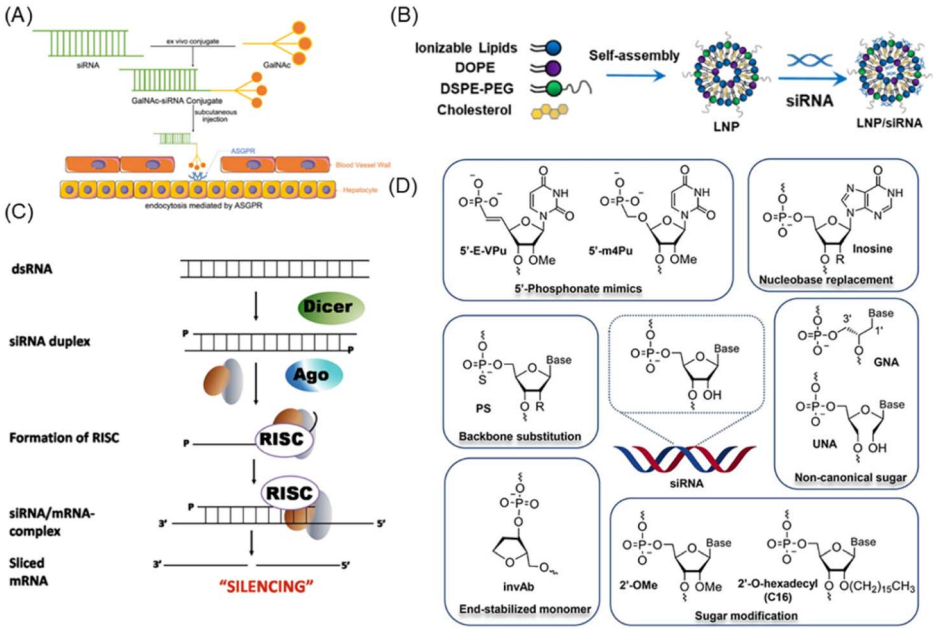


Fig. 5 siRNA delivery strategies and mechanisms (A) schematic of trivalent ligands with terminal GalNAc moieties covalently conjugated to siRNA (B) liposomal systems for siRNA therapeutic delivery (C) mechanism of siRNA-mediated gene silencing (D) representative siRNA designs and chemical modifications in clinical development. (A) Adapted from ref. 89. Licensed under a Creative Commons CC BY-NC 4.0 License. (B) Reproduced with permission from ref. 90. Copyright 2023, Elsevier (C) Reproduced with permission from ref. 97. Copyright 2018, Springer. (D) Reproduced with permission from ref. 106. Copyright 2024, Elsevier.

industrial paradigm of "chemical modification + liver-targeted delivery". For example, Nair *et al.* demonstrated that appropriately protected synthetic GalNAc ligands are compatible with solid-phase oligonucleotide synthesis, thereby providing an efficient manufacturing process for rapid identification and optimization of lead candidates.[91] Optimized designs of multivalent GalNAc–conjugated siRNAs can elicit potent RNAi-mediated gene silencing in hepatocytes both *in vitro* and *in vivo* without the need for additional delivery vehicles. Divesiran (SLN124), a liver-targeted GalNAc–siRNA conjugate, exemplifies this approach by silencing the negative regulator of hepcidin production (TMPRSS6) to enhance hepatic hepcidin synthesis and elevate plasma levels, thereby modulating hematocrit in polycythemia vera (PV).[92]

Following administration, LNP–siRNA is primarily internalized into hepatocytes through ApoE coating and subsequent interaction with LDLR, whereas GalNAc–siRNA undergoes ASGPR-mediated endocytosis. After particles or conjugates enter early endosomes, endosomal escape represents a critical bottleneck for pharmacological activity: LNPs rely on ionizable lipids that disrupt membranes under acidic conditions to facilitate escape, while GalNAc–siRNAs depend on limited spontaneous leakage into the cytoplasm.[93,94] Once in the cytoplasm, double-stranded siRNA is incorporated into RISC: Ago2 recognizes and retains the thermodynamically less stable strand as the guide strand (whose 5′ phosphate pairs with the MID domain), while the passenger strand is either cleaved or displaced. The guide strand then uses its seed region (nt 2–8) to search for complementary sequences and establishes full-length base pairing with the target mRNA.[95] Ago2 subsequently cleaves the mRNA backbone between the 10th and 11th nucleotides relative to the guide strand's 5′ end, with the cleavage products degraded by cellular ribonucleases, while RISC undergoes multiple catalytic cycles to mediate gene silencing (Fig. 5C). Meanwhile, partial complementarity may elicit miRNA-like off-target effects, most commonly *via* seed pairing at the 3′ UTR.[96] To mitigate this, modern siRNA design employs site-selective base and sugar modifications, together with thermodynamic asymmetry optimization, to suppress off-target activity and extend half-life. Benefiting from the efficient turnover of the Ago2–RISC complex with target mRNAs in hepatocytes, coupled with enhanced chemical stability, clinical applications have now achieved durable gene silencing with quarterly to semiannual dosing regimens, offering a practical therapeutic strategy for long-term management of chronic diseases.

2.3.2. Clinical applications of siRNA. Over the past decade, siRNA therapeutics have established a clear clinical spectrum in liver-targeted diseases, demonstrating quantifiable efficacy and diversified dosing regimens. For example, patisiran, administered by intravenous infusion of LNP every three weeks,[97] showed in the APOLLO trial that after 18 months of treatment, patients exhibited significantly better modified Neuropathy Impairment Score (mNIS+7) outcomes compared with placebo, with a considerable proportion achieving improvement from baseline. In parallel, sustained and rapid reductions in serum TTR levels confirmed the disease-modifying potential of LNP–siRNA in protein deposition disorders.[98] Targeting the same pathway, vutrisiran employs GalNAc conjugation for subcutaneous administration once every three months;[99] in the

HELIOS-A study, it met the primary endpoint of mNIS+7 improvement at 9 months, while also achieving statistically and clinically meaningful benefits across multiple key secondary endpoints, including the Norfolk QOL-DN quality-of-life score and the 10-meter walk test, thereby validating the feasibility of the "ligand conjugation plus extended dosing interval" paradigm. In the field of metabolic and genetic diseases, givosiran, approved for acute hepatic porphyria (AHP), significantly reduced the annualized attack rate (AAR), providing an effective strategy for long-term disease management.[100] Likewise, lumasiran, developed for primary hyperoxaluria type 1 (PH1), achieved an average ~65% reduction in 24-hour urinary oxalate levels within 3–6 months in the ILLUMINATE-A study, with consistent efficacy across different renal function subgroups.[101] Furthermore, inclisiran, a PCSK9-targeting siRNA, is administered with a loading regimen at 0 and 3 months followed by maintenance dosing every 6 months; the ORION-10/11 trials demonstrated an approximate 50% reduction in LDL-C at day 510, thus establishing a "twice-yearly dosing" paradigm for common chronic conditions.[102]

The success of siRNA therapeutics is fundamentally supported by an integrated engineering framework encompassing "chemical modification, receptor–ligand targeting, and endosomal escape".[103,104] At the sequence level, the prevailing clinical strategy adopts alternating patterns of 2′-OMe and 2′-F ribose modifications, combined with limited terminal PS linkages.[105] This configuration enhances nuclease resistance and plasma stability while markedly attenuating innate immune recognition. Notably, the incorporation of 2′-OMe at U-rich motifs effectively suppresses TLR7/8-mediated cytokine release, representing a classical approach to reducing immunostimulatory reactivity (Fig. 5D).[106] To further improve Ago2 loading and prolong *in vivo* exposure, the guide strand 5′ terminus is frequently modified with a 5′-(*E*)-vinylphosphonate group, which mimics the natural 5′-phosphate, strengthens binding to the MID domain, and thereby enhances pharmacological activity and tissue retention. In addition, site-specific modifications within the seed region (nt 2–8), combined with thermodynamic asymmetry design, mitigate miRNA-like off-target effects and hepatotoxicity, as exemplified by Alnylam's Enhanced Stabilization Chemistry (ESC) and Enhanced Stabilization Chemistry Plus (ESC+) platforms. At the delivery level, early-generation LNP systems (*e.g.*, the MC3 ionizable lipid used in patisiran) rely on lipid protonation and phase transition upon endosomal acidification to facilitate endosomal escape.[107–109] In contrast, triantennary GalNAc conjugates exploit ASGPR-mediated endocytosis in hepatocytes, thereby enabling subcutaneous administration, liver-specific uptake, and quarterly to biannual dosing intervals. This strategy has been successfully validated in multiple approved products, including givosiran, lumasiran, vutrisiran, and inclisiran.

2.4. Aptamer

2.4.1. Mechanisms of action of aptamers. Aptamers are single-stranded oligonucleotides selected from synthetic random DNA or RNA libraries *via* the *in vitro* SELEX process,[110] capable of binding proteins, small molecules, cells, and even whole pathogens with high affinity and specificity, and are therefore often termed "chemical antibodies." Compared with antibodies, aptamers display several advantages: (1) simplified selection, as they can be generated rapidly without animal or cell systems under non-physiological conditions—for example, the SARS-CoV-2 aptamer CoV2-RBD-1C was identified after only 12 selection rounds;[111] (2) ease of synthesis and scalability, since they can be produced at high purity and low cost through solid-phase synthesis; (3) programmability and flexible chemical modification, enabling the precise introduction of functional groups, linkers, or regulatory elements;[112–114] (4) low immunogenicity, as they generally do not elicit strong adaptive immune responses; (5) a broad target spectrum, allowing them to bind nearly any molecule with an accessible binding site, including non-immunogenic targets; and (6) small molecular size, which reduces steric hindrance and enhances tissue penetration.[115]

Aptamers exhibit dual mechanisms in therapeutic applications, the first being their role as targeted delivery vehicles. Through covalent or noncovalent conjugation with therapeutic agents—including small-molecule drugs, toxins,[116] radionuclides, siRNA/ASO,[117] and proteins[118]—aptamers enable precise delivery. In 2009, Huang *et al.* first introduced the concept of aptamer–drug conjugates (ApDCs) (Fig. 6A), which has since become the most widely adopted strategy for utilizing aptamers as delivery tools.[119] For instance, Bagalkot *et al.*[120] demonstrated the physical conjugation of an RNA aptamer with doxorubicin (DOX) *via* intercalation of the anthracyclic ring, enabling targeted delivery to prostate-specific membrane antigen (PSMA)-positive cancer cells, while Li *et al.*[121] designed a cathepsin B-responsive dipeptide linker (NucA-PTX) that releases paclitaxel intracellularly upon enzymatic cleavage, thereby achieving tumor-selective drug delivery (Fig. 6B). The hydrophilic backbone of aptamers enhances the solubility of such conjugates, promotes tumor accumulation, improves therapeutic efficacy, and reduces systemic toxicity. During the delivery process, aptamers specifically bind to receptors on the surface of target cells and undergo receptor-mediated endocytosis, after which active drugs are released within endosomal/lysosomal microenvironments (*e.g.*, low pH, reductive conditions, or enzymatic cleavage) or *via* endosomal escape mechanisms,[122] thereby exerting intracellular therapeutic effects while markedly reducing off-target toxicity.[123] Moreover, aptamers can also serve as functional carriers for photosensitizers. For example, Tan and colleagues[124] developed giant membrane vesicles (GMVs) co-loaded with the aptamer AS1411, the photosensitizer TMPyP4, and the photothermal agent ICG, using chol-Sgc8 aptamer for PTK7 targeting (Fig. 6C), which demonstrated enhanced cytotoxicity and therapeutic efficacy against CCRF-CEM cells.

The second therapeutic mechanism of aptamers lies in their direct function as modulators (antagonists or agonists), whereby they regulate the biological activity of target proteins through binding to specific functional domains. Aptamers can exploit complex secondary structures such as G-quadruplexes to precisely recognize protein epitopes: these structures are formed by guanine-rich sequences that generate planar G-

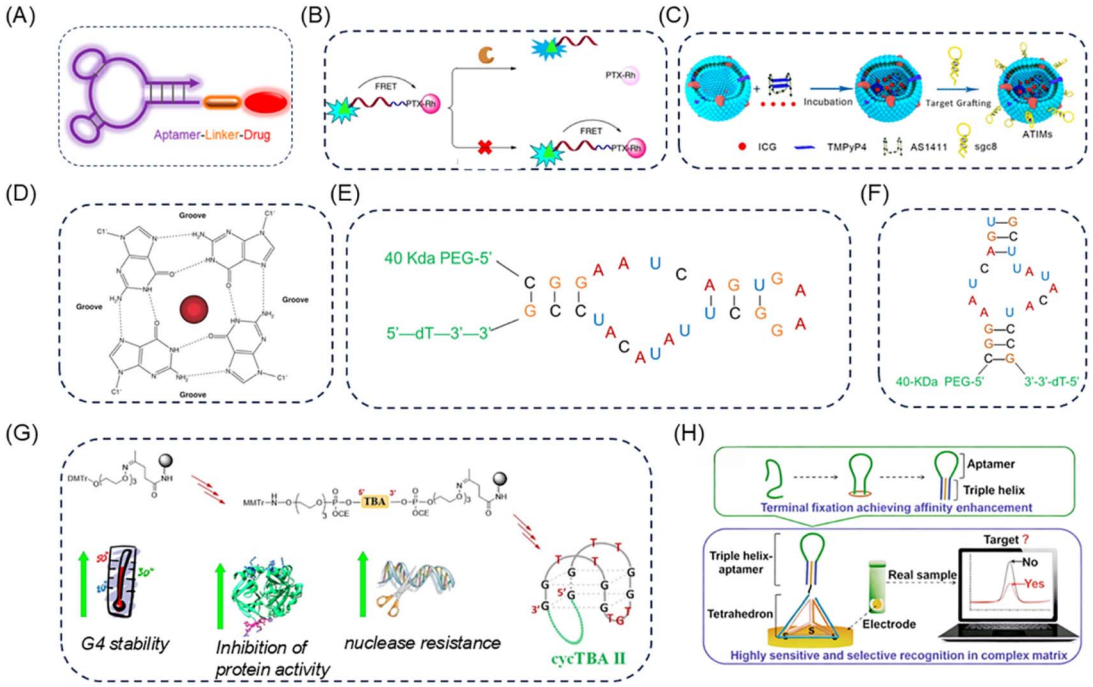


Fig. 6 Representative designs and applications of aptamer-based therapeutics and structural modifications (A) composition of ApDCs; (B) schematic of a water-soluble nucleotide aptamer–paclitaxel conjugate for ovarian cancer-specific targeting; (C) biomimetic vesicle-based carrier for targeted drug delivery and combined photodynamic/photothermal therapy; (D) arrangement of guanine bases in G-quadruplex with centrally coordinated metal ion (hydrogen bonds shown as dashed lines); (E) structural representation of Pegaptanib; (F) structural representation of Avacincaptad pegol; (G) mechanism of cyclization-based tuning of thrombin-binding aptamer properties; (H) schematic of engineered aptamer with affinity enhancement *via* triplex-based terminal fixation. (A) Reproduced with permission from ref. 119. Copyright 2015, American Chemical Society. (B) Reproduced with permission from ref. 121. Copyright 2017, Springer Nature. (C) Reproduced with permission from ref. 124. Copyright 2019, American Chemical Society. (D) Reproduced from ref. 125. Licensed under a Creative Commons CC BY-NC 2.0 License. (E) (F) Created by the authors using Microsoft PowerPoint. (G) Reproduced with permission from ref. 142. Copyright 2020, American Chemical Society (H) Reproduced with permission from ref. 143. Copyright 2019, American Chemical Society.

quartets *via* Hoogsteen hydrogen bonding, which further stack into stable G-quadruplexes stabilized by monovalent cations within the central channel (Fig. 6D).[125] The G-quadruplex provides a rigid, negatively charged interface that enables high-affinity and high-specificity recognition of positively charged surface regions or functional pockets of proteins, thereby directly interfering with protein function. As antagonists, aptamers block the interaction of target proteins with their natural ligands or receptors to inhibit downstream signaling. For example, the G-quadruplex-forming aptamer AS1411 binds to nucleolin, which is overexpressed on cancer cell surfaces, thereby suppressing its function and inducing apoptosis and cell cycle arrest.[126] Conversely, as agonists, aptamers activate signaling by binding to and stabilizing active conformations of their targets. Although currently limited in number, reported examples include RNA aptamers against HER3,[127] CD28,[128] OX40,[129] 4-1BB,[130] CD40,[131] VEGFR-2,[132] and the insulin receptor (IR).[133]

2.4.2. Clinical applications of aptamer. Aptamer therapeutics have continued to make significant progress in clinical translation, with two drugs to date approved by the U.S. FDA: pegaptanib (Macugen) and avacincaptad pegol (Izervay). Pegaptanib is an RNA aptamer targeting the VEGF165 isoform for the treatment of neovascular (wet) age-related macular degeneration (AMD). Its sequence incorporates 2′-F-modified pyrimidine nucleotides and 2′-OMe modified purine nucleotides, with a 40 kDa polyethylene glycol (PEG) moiety conjugated at the 3′-end to markedly enhance molecular stability and *in vivo* half-life (Fig. 6E). Delivered *via* intravitreal injection, pegaptanib became the first FDA-approved aptamer drug, and its successful clinical translation validated the feasibility and therapeutic potential of aptamers as novel medicines.[134] In contrast, avacincaptad pegol represents the latest breakthrough in the treatment of the late-stage dry AMD subtype, geographic atrophy (GA). It is a 28-nucleotide PEGylated RNA aptamer designed to improve pharmacokinetic performance (Fig. 5F). Mechanistically, avacincaptad pegol binds specifically to complement component C5, blocking its cleavage into the proinflammatory mediator C5a and the membrane attack complex precursor C5b, thereby effectively inhibiting the complement cascade and mitigating retinal cell damage. Clinical studies have demonstrated that intravitreal administration of avacincaptad pegol significantly slows guanine–adenine (GA) lesions progression while exhibiting favorable systemic safety and tolerability.[135]

2.4.3. Chemical modifications and conformational optimization. Compared with other nucleic acid drugs, the number of aptamers in clinical use remains limited, largely due to their

susceptibility to nuclease degradation and short *in vivo* half-life, making chemical modifications essential for druggability. Common strategies to enhance aptamer stability and nuclease resistance include sugar modifications, backbone modifications, and terminal capping.[136] Sugar modifications such as 2′-F, 2′-OMe, and 2′-amino (2′-NH_2) substitutions for the 2′-OH of RNA effectively improve resistance to enzymatic degradation.[137] In backbone modifications, PS and phosphorodithioate (PS_2) substitutions replace oxygen atoms with sulfur, thereby enhancing nuclease resistance and prolonging half-life, although they may affect binding affinity. For example, Tan and colleagues[138] fully substituted the CD71-targeting aptamer XQ-2d with PS linkages to generate S-XQ-2d, which showed markedly improved plasma stability and extended circulation half-life in mice. At the 3′ end, addition of inverted dT or PEG protects against exonuclease degradation, while PEGylation also increases molecular weight, reduces renal clearance, minimizes nonspecific binding, and improves solubility. LNAs, in which a methylene bridge links the 2′-O and 4′-C positions, significantly enhance binding affinity and thermal stability.[139,140] Other approaches include Spiegelmers (mirror-image oligonucleotides), composed of L-nucleotides with superior biological stability but requiring selection against the enantiomeric target, thereby limiting applications;[141] circularization, in which aptamers are covalently linked end-to-end to form closed loops, improving structural rigidity, nuclease stability, and functional activity—for instance, Riccardi *et al.*[142] employed oxime ligation or CuAAC to cyclize the thrombin-binding aptamer (TBA), enhancing both stability and anticoagulant activity (Fig. 6G); inter-strand locking, achieved by incorporating cross-linkers or modified bases at key positions within G-quadruplexes to stabilize the active conformation; and optimization of G-tracts or base modifications to maximize binding performance. Furthermore, Tan and colleagues[143] reported the use of triplex structures to constrain aptamer termini and reduce flexibility, resulting in nearly a tenfold increase in affinity for an anti-lysozyme aptamer (Fig. 6H).

2.5. mRNA

Messenger RNA (mRNA) represents a versatile platform for nucleic acid therapeutics. Beyond the remarkable success of mRNA vaccines, its applications have expanded to encompass diverse therapeutic modalities, including protein replacement therapy, gene therapy, and regenerative medicine. Therefore, this section provides an overview of the major therapeutic strategies based on mRNA technology, highlighting their underlying mechanisms and recent clinical progress.

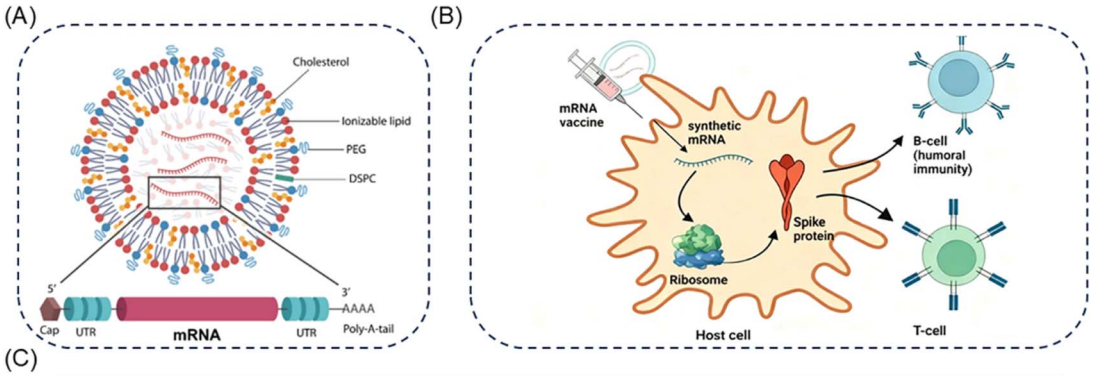
2.5.1. mRNA vaccine

2.5.1.1. Mechanism of action of mRNA vaccine. The concept of using mRNA as a vaccine substrate dates back to 1990, when Wolff *et al.* demonstrated for the first time that intramuscular injection of mRNA/DNA into mice could successfully express reporter proteins, thereby proving the feasibility of "*in vivo* translation of messenger RNA" and laying the foundation for mRNA therapeutics.[144,145] However, the intrinsic immunogenicity and instability of mRNA long hindered its clinical development. Around 2005, Karikó and Weissman introduced nucleoside modifications (*e.g.*, Ψ and 1-methylpseudouridine) that markedly reduced innate immune activation and enhanced translational efficiency, a breakthrough widely recognized as the turning point for the platform.[146] This work, which paved the way for COVID-19 mRNA vaccines, was honored with the 2023 Nobel Prize in Physiology or Medicine. In 2020, mRNA vaccines BNT162b2 and mRNA-1273 were granted emergency use authorization and subsequently full approval worldwide, marking the successful transition of mRNA vaccines "from concept to industry."[147,148]

The fundamental composition of mRNA vaccines consists of two major components: the nucleic acid sequence itself and the delivery system. The mRNA molecule typically contains a 5′-cap structure, untranslated regions (UTRs), an open reading frame (ORF), and a 3′ polyadenylated tail (poly-A tail), which collectively ensure intracellular stability and efficient translation. To overcome nuclease degradation and the cell membrane barrier, mRNA is encapsulated within lipid nanoparticles (LNPs) for delivery. A typical LNP is composed of four essential constituents: (i) ionizable lipids, which electrostatically complex with negatively charged mRNA under acidic conditions and facilitate endosomal release; (ii) cholesterol, which enhances particle stability and membrane fluidity; (iii) structural phospholipids (*e.g.*, DSPC), which maintain bilayer integrity; and (iv) PEG–lipids, which form a hydrophilic corona on the particle surface to reduce plasma protein adsorption and prolong circulation (Fig. 7A).[149]

Following intramuscular injection, LNP-encapsulated mRNA forms a transient local depot, with a portion of particles draining to regional lymph nodes (Fig. 7B).[150,151] The combination of mRNA with ionizable lipids triggers mild innate immune signalling, recruiting antigen-presenting cells (APCs) such as dendritic cells (DCs). Upon uptake of LNPs by myocytes and APCs, endosomal acidification leads to protonation of ionizable lipids, disrupting membrane integrity and promoting cytosolic release of mRNA, which is subsequently translated by ribosomes. The resulting antigens undergo processing through the endoplasmic reticulum-Golgi network, appearing either as secreted proteins or membrane-anchored forms. Secreted antigens are taken up by APCs and presented *via* the MHC II pathway to activate $CD4^+$ T cells (particularly T follicular helper cells, Tfh), driving germinal center reactions, class switching, and affinity maturation. In parallel, membrane-associated or endogenously synthesized antigens are processed by the immunoproteasome–TAP complex and presented *via* MHC I, thereby activating $CD8^+$ T cells to mount cytotoxic responses.[152]

These processes generate neutralizing antibodies from 9 long-lived plasma cells in the bone marrow, together with memory B and T cells that establish durable protection. Antigen expression typically peaks within 24–48 h and declines as mRNA is degraded; booster doses rapidly expand the memory pool and significantly increase antibody titers. Ultimately, mRNA is degraded by nucleases, and LNPs are cleared through the hepatobiliary pathway. Importantly, mRNA vaccines do not enter the nucleus and pose no risk of genomic integration, while



(C)

Vaccine	Indication	Clinical Progresss
mRESVIA (mRNA-1345)	Respiratory Syncytial Virus (RSV)	FDA approved in 2024 for ≥60 years; expanded in 2025 to high-risk adults aged 18–59
mRNA-1010	Seasonal Influenza	Phase III trial, showing superiority (26.6%) over a licensed standard-dose influenza vaccine; regulatory submission ongoing
mRNA-1647	Cytomegalovirus	Phase III CMVictory (P301) trial ongoing
RABV-G mRNA	Rabies Virus	Phase I clinical trial
mRNA-4157 (V940)	Cancer immunotherapy (melanoma, NSCLC)	Phase III trial in combination with pembrolizumab, expanded from melanoma to multiple cancers

Fig. 7 Mechanisms and clinical development of mRNA vaccines (A) schematic illustration of an mRNA vaccine; (B) mechanism of action of mRNA-based vaccines; (C) clinical progress of mRNA vaccines during 2024–2025. (A) Reproduced from ref. 149. Licensed under a Creative Commons CC BY-NC 4.0 License. (B) Reproduced from ref. 150. Licensed under a Creative Commons CC BY-NC 4.0 License.

common adverse events are generally self-limited, consisting of local erythema/swelling and transient systemic symptoms.[153,154]

2.5.1.2. Clinical progress of mRNA vaccine. In recent years (2024–2025), the mRNA platform has transitioned from a single emergency response against COVID-19 toward a "multi-disease, routine" development paradigm, achieving multiple breakthroughs in clinical settings. In the respiratory field, Moderna's RSV vaccine mRESVIA (mRNA-1345) was approved by the FDA in 2024 for adults aged ≥60 years,[155] and in 2025 its indication was further expanded to high-risk adults aged 18–59, reflecting the establishment of a relatively mature regulatory pathway for this indication.[156] In the same year, its seasonal influenza candidate mRNA-1010 demonstrated a 26.6% superiority over a licensed standard-dose influenza vaccine in a phase III study involving 40 800 participants,[157] and based on these results the company initiated regulatory submission discussions, marking the first clear clinical evidence of superiority for mRNA vaccines in influenza.[158] Beyond respiratory infectious diseases, Moderna's cytomegalovirus (CMV) vaccine mRNA-1647 is advancing in the phase III CMVictory (P301) trial, with primary endpoints focusing on seroconversion prevention, safety, and immunogenicity, representing one of the first "routine" mRNA vaccines with registration potential following COVID-19. In addition, an RABV-G mRNA vaccine for rabies virus glycoprotein has entered phase I clinical evaluation.

In oncology, progress is being driven by personalized neoantigen vaccines. mRNA-4157 (V940) in combination with pembrolizumab has advanced from melanoma into multiple phase III trials in NSCLC and other indications,[159] with early follow-up data demonstrating sustained benefit in recurrence and metastasis outcomes for high-risk melanoma, thereby providing clinical evidence of durable efficacy for the "vaccine plus checkpoint inhibitor" strategy (Fig. 7C).[160] In parallel with these clinical efforts, formulation and delivery technologies are addressing critical limitations. Several studies have confirmed that lyophilized mRNA–LNP formulations can maintain physicochemical and immunological activity at 4–25 °C for extended periods, offering a promising means to reduce cold-chain dependence.[161] Meanwhile, organ-selective LNPs designed for extrahepatic delivery—such as the selective organ targeting (SORT) strategy, which incorporates defined fractions of supplemental lipids to tune biodistribution, and its derivatives—have achieved programmable biodistribution to tissues such as the lung and kidney through advances in materials and formulation engineering, opening a "targeted delivery" window for next-generation prophylactic and therapeutic mRNA agents.[162]

Overall, the recent progress of mRNA is characterized by three parallel dimensions—indication expansion, accumulation of registrational evidence, and advances in delivery/stability engineering—which together consolidate the public health value of the vaccine platform while providing a pathway and toolkit for the clinical translation of therapeutic mRNA.

2.5.2. Protein replacement therapy. Protein replacement therapy is a therapeutic strategy designed to replace or supplement deficient protein function, aiming to correct disease phenotypes caused by gene mutations that lead to protein loss or dysfunction.[163] The concept of mRNA-based

protein replacement therapy centers on *in vivo* translation, whereby exogenous mRNA serves as a "temporary genetic instruction" to direct the patient's own cells—typically hepatocytes or myocytes—to synthesize the desired therapeutic protein, thereby restoring or compensating for the function of the endogenous counterpart.[164] In this approach, chemically modified mRNA is encapsulated within LNPs and delivered either systemically (*e.g.*, *via* intravenous injection) or locally to the target tissue, where it is taken up by the recipient cells.[165] Once in the cytoplasm, the mRNA is translated by ribosomes into a functional protein, which either acts intracellularly or is secreted into the circulation to perform its physiological function, thus compensating for the protein deficiency caused by the genetic defect. Compared with conventional protein- or DNA-based therapies, the mRNA platform offers several distinct advantages: (i) mRNA bypasses the need for nuclear delivery and transcription, enabling more efficient protein expression; (ii) mRNA does not integrate into the host genome, providing superior genetic safety; and (iii) its transient expression profile minimizes the risks of insertional mutagenesis and oncogenic transformation, thereby enhancing overall therapeutic safety.

The use of mRNA to provide functional copies of missing or dysfunctional proteins offers a highly promising strategy for the treatment of monogenic metabolic disorders. By encapsulating mRNA encoding functional enzymes within lipid nanoparticles (LNPs) and delivering them to target cells, it is possible to restore the activity of key metabolic pathways *in vivo*, thereby correcting long-term metabolic impairments. For example, Ding *et al.* developed a pseudouridine (Ψ)-modified codon-optimized mRNA–LNP formulation encoding human methylmalonyl-CoA mutase (hMUT), the enzyme most frequently mutated in methylmalonic acidemia (MMA). This system achieved efficient protein expression and remarkable metabolic improvement in murine models, effectively reversing the pathological phenotype of MMA; the therapy has now advanced to clinical evaluation.[166] Similarly, Koeberl D. *et al.* reported the interim results of a phase I/II clinical trial for propionic acidemia (PA), which systematically evaluated the safety and efficacy of mRNA-3927—a dual mRNA therapeutic candidate encoding PCCA and PCCB—thereby providing proof-of-concept evidence for precise treatment of biallelic enzymatic deficiencies.[167] In addition, Yamazaki K. *et al.* developed an engineered hOTC-mRNA/LNP formulation (encoding human ornithine transcarbamylase, hOTC) that demonstrated a significant dose-dependent therapeutic response in an ornithine transcarbamylase deficiency (OTCD) mouse model, markedly improving survival rates.[168] These findings collectively underscore the clinical potential of mRNA-based protein replacement therapy for rare inherited metabolic disorders.

2.5.3. Gene therapy and regenerative medicine. Gene editing and regenerative medicine represent two additional important frontier applications of mRNA technology. The core concept of gene editing lies in harnessing the transient expression capacity of mRNA to enable potent yet controllable *in vivo* production of gene-editing tools such as CRISPR–Cas9. By co-delivering mRNA encoding the Cas9 protein together with a single guide RNA (sgRNA) that directs sequence-specific recognition and cleavage of the target gene locus, precise gene knockout, correction, or insertion can be achieved without the need for viral vectors.[169] In the cytoplasm, ribosomes translate the Cas9 mRNA into protein, which then assembles with the sgRNA to form an active ribonucleoprotein complex capable of introducing double-stranded breaks at the desired DNA site. Because both mRNA and Cas9 exist only transiently, this strategy substantially reduces the risks of off-target editing and long-term immune activation, providing a safer and more controllable alternative to viral vector-based systems that mediate persistent Cas9 expression. Gillmore J. D. *et al.* reported the first *in vivo* gene-editing therapy, NTLA-2001, in a phase I clinical trial.[170] This therapy utilizes lipid nanoparticles (LNPs) to co-deliver mRNA encoding *Streptococcus pyogenes* Cas9 (SpCas9) together with an sgRNA targeting the transthyretin (TTR) gene, thereby achieving precise knockout of the TTR gene in patients' hepatocytes. The results demonstrated a dose-dependent and sustained reduction in circulating pathogenic transthyretin protein levels, providing direct clinical evidence that the mRNA–LNP platform can safely and efficiently achieve therapeutic gene editing *in vivo*.

In the field of regenerative medicine, mRNA technology promotes tissue repair and regeneration by transiently inducing the expression of regenerative and pro-healing factors. For instance, Zangi L. *et al.* first demonstrated that synthetic mRNA can drive efficient *in vivo* expression of vascular endothelial growth factor A (VEGF-A).[171] When chemically modified VEGF-A mRNA was directly injected into the myocardium of a mouse model of myocardial infarction, it markedly induced the differentiation of cardiac progenitor cells into endothelial cells, stimulated the formation of functional neovasculature, and significantly improved cardiac performance. This pioneering study established both the conceptual and experimental foundation for the use of mRNA in regenerative medicine, highlighting its broad potential in promoting tissue repair and organ regeneration.

2.6. Nucleic acid nanostructures

The aforementioned classes of nucleic acid therapeutics (ASOs, siRNAs, miRNAs, mRNAs, and aptamers) primarily rely on sequence design and chemical modification strategies to achieve precise regulation of gene expression and therapeutic intervention. With the rapid advances in structural biology and nanotechnology, researchers have further sought to engineer nucleic acids in the spatial dimension, giving rise to a new class of artificial nucleic acid nanostructures characterized by high programmability and controllable self-assembly.[172] These structures exploit the stringent base-pairing principles of nucleic acids (A–T/U and C–G) to achieve sequence-specific recognition among designed segments.[173] Through precise intra- or intermolecular hybridization, they can assemble into complex higher-order architectures at the nanoscale, thereby endowing the system with well-defined geometries and tunable functionalities. Representative examples of such nucleic acid nanostructures include spherical nucleic acids and DNA origami, which have emerged as versatile platforms for

structural innovation and functional modulation in nucleic acid therapeutics.[174]

2.6.1. Spherical nucleic acids. Spherical nucleic acids (SNAs) refer to three-dimensional nucleic acid shell structures formed by the high-density arrangement of oligonucleotides on the surface of nanoparticles, whose cores are typically composed of inorganic materials such as gold, silver, or silica. The concept was first proposed and realized by Professor Chad A. Mirkin in 1996 (Fig. 8A).[175] In his pioneering design, thiol-modified DNA strands were covalently anchored onto the surface of gold nanoparticles (approximately 13 nm in diameter) *via* Au–S bonds, forming a densely packed and highly ordered nucleic acid corona. This "hard-core/soft-shell" architecture not only markedly enhances the structural stability of nucleic acids but also endows SNAs with physicochemical properties distinct from those of their linear counterparts. For instance, SNAs exhibit exceptional cellular uptake capability and can efficiently enter a wide range of cells without the need for transfection agents, thereby overturning the long-standing notion that nucleic acids inherently struggle to cross cellular membranes.[176]

SNAs exhibit distinctive structural and functional advantages.[177] First, their core–shell architecture endows the system with exceptional physicochemical stability.[178] The densely packed nucleic acid shell effectively protects the oligonucleotides from nuclease degradation, thereby significantly extending their circulation half-life in complex biological environments. Meanwhile, the inorganic nanoparticle core (such as gold nanoparticles) provides robust structural support, ensuring the overall integrity and reproducibility of the construct. Second, the most striking feature of SNAs lies in their remarkably high cellular uptake efficiency.[179] Unlike conventional linear nucleic acids that require transfection reagents to enter cells, SNAs can be actively and efficiently internalized by a wide range of cell types—including traditionally hard-to-transfect primary cells—*via* clathrin-mediated endocytosis, thus overcoming one of the major barriers in nucleic acid drug delivery. Moreover, the high-density oligonucleotide shell of SNAs generates a pronounced multivalent effect, which not only enhances their hybridization affinity toward complementary sequences but also provides a versatile platform for molecular functionalization.[180] By co-conjugating different types of functional nucleic acids (*e.g.*, siRNA or aptamers) or chemical moieties on the same nanoparticle surface, SNAs can achieve targeted delivery, synergistic therapy, and stimuli-responsive behavior, offering unprecedented freedom in molecular design and programmability in biological function.

Building upon these advantages, SNAs have demonstrated significant value in the design and delivery of nucleic acid therapeutics. In terms of delivery, SNAs can be efficiently internalized by a wide variety of cell types without the need for transfection agents, thereby avoiding the cytotoxicity and immunogenicity commonly associated with traditional nonviral vectors. For example, Jie Li *et al.* proposed an interface engineering strategy based on a tetrahedral DNA framework (tDF) to construct a novel DNA framework spherical nucleic acid (tDF-

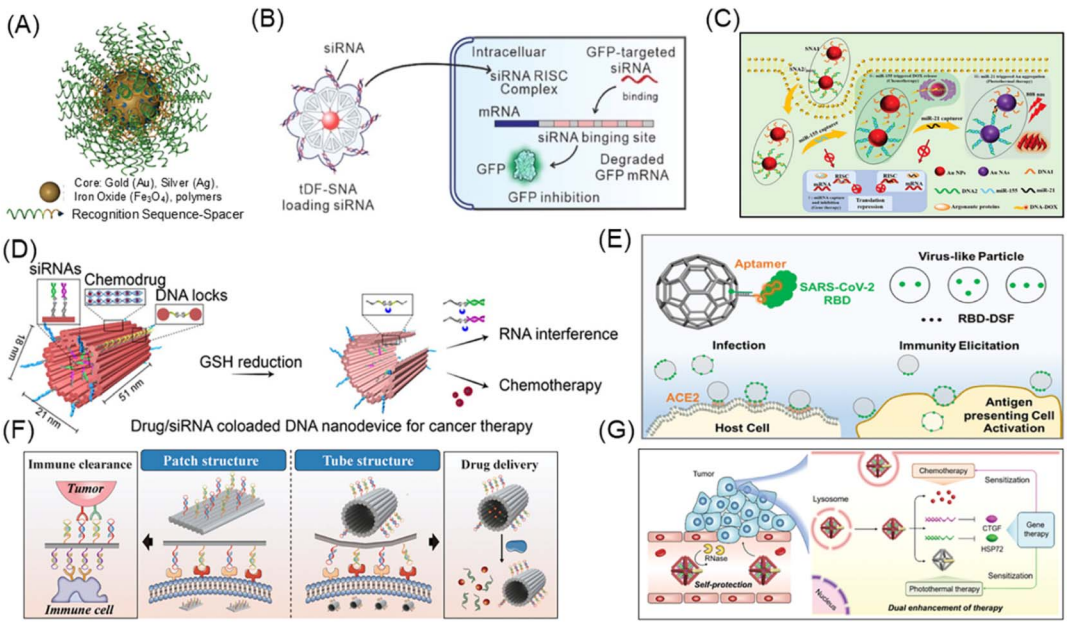


Fig. 8 Applications of nucleic acid nanostructures in nucleic acid therapeutics. (A) Structural schematic of SNAs. (B) SNAs enhance siRNA delivery efficiency. (C) Multimodal therapeutics enabled by SNAs. (D) DNA origami as a programmable platform for drug delivery. (E) DNA origami for elucidating how ligand organization influences viral infection and immune activation. (F) DNA origami modulates tumor-targeting selectivity for immune clearance and therapeutic delivery. (G) DNA origami-based platforms for multimodal therapy. (A) Reproduced with permission from ref. 176. Copyright 2012, American Chemical Society. (B) Reproduced with permission from ref. 182. Copyright 2025, Wiley-VCH. (C) Reproduced with permission from ref. 185. Copyright 2022, Wiley-VCH. (D) Reproduced with permission from ref. 198. Copyright 2021, Wiley-VCH. (E) Reproduced with permission from ref. 199. Copyright 2022, American Chemical Society. (F) Reproduced with permission from ref. 200. Copyright 2024, American Chemical Society. (G) Reproduced with permission from ref. 201. Copyright 2021, Wiley-VCH.

SNA).[181] In this design, siRNA-loaded tDFs were precisely anchored onto gold nanoparticle surfaces through Au–S bonds, forming corona-like spike structures with flexible conformations around the nanoparticle core. This architecture led to a 1–2 order of magnitude increase in siRNA delivery efficiency and approximately a twofold enhancement in specific gene-silencing activity (Fig. 8B). In addition, SNAs with lipid or polymeric cores (such as liposomal SNAs and polymer-core SNAs) have been extensively employed for the delivery of mRNA and ASOs, exhibiting improved serum stability and prolonged *in vivo* circulation times.[182] Furthermore, the three-dimensional spherical topology of SNAs confers pronounced multivalency and cooperative recognition capabilities.[183] By arranging multiple functional oligonucleotides with precise spatial control on a single nanoparticle surface, SNAs can simultaneously mediate multivalent molecular recognition and synergistic regulation within a unified nanoscale platform. On this basis, Wang *et al.* developed an intelligent SNA system in which two antisense oligonucleotides complementary to the oncogenic miRNAs miR-21 and miR-155 were covalently grafted onto gold nanoparticles. In addition, the chemotherapeutic drug DOX and a photosensitizer were hybridized onto the antisense strands. This multifunctional SNA could simultaneously capture target miRNAs and release both the photosensitizer and DOX in a controlled manner, thereby achieving combined gene, photodynamic, and chemotherapeutic effects within a single nanosystem (Fig. 8C).[184] At the clinical translation level, SNA technology has begun to move toward practical application. The SNA platform developed by Exicure Inc. (including AST-008) has been employed as a Toll-like receptor 9 (TLR9) agonist for immunotherapy and is currently under clinical investigation for the treatment of melanoma and breast cancer.[185] Moreover, the SNA-based drug XCUR17, designed for psoriasis therapy, demonstrated favorable safety and significant gene-silencing efficacy in a Phase I clinical trial.[186] Collectively, these studies highlight that SNAs not only exhibit superior performance in nucleic acid delivery and tissue penetration, but also hold broad potential in cancer therapy, immunomodulation, and the treatment of inflammatory diseases.

2.6.2. DNA origami. The DNA origami technique represents another milestone innovation in the field of nucleic acid nanostructures.[187] Its core principle is based on the programmable Watson–Crick base-pairing rules (A–T and C–G), whereby a long single-stranded DNA "scaffold" is precisely hybridized with hundreds of short complementary "staple strands." Through this highly specific hybridization process, the scaffold strand can spontaneously fold along a predesigned path at the nanoscale, forming structures with well-defined geometries and spatial configurations. This concept was first proposed and experimentally demonstrated by Paul W. K. Rothemund in 2006, in a landmark paper published in Nature.[188] In this pioneering work, more than 200 short oligonucleotides were designed to fold a single-stranded DNA molecule into a variety of two-dimensional shapes—such as smiley faces and stars—thereby providing the first proof-of-concept for programmable DNA folding. Rothemund's work not only established the term "DNA origami" but also marked a conceptual transition of nucleic acids from passive carriers of genetic information to programmable structural and functional materials. With advances in design strategies and synthesis methods, DNA origami rapidly evolved from two-dimensional patterns to complex three-dimensional architectures.[189] In 2009, Douglas *et al.* reported in Nature a computer-aided design (CAD) approach for three-dimensional DNA origami, which enabled the construction of tubular, cubic, and box-like nanostructures with precisely controlled dimensions and morphologies.[190] This work provided a standardized design toolkit and a general framework for DNA-based nanofabrication. Subsequently, researchers have developed dynamic and stimuli-responsive DNA origami systems, in which deformable hinges, trigger strands, or pH/ion-responsive modules are integrated into the structures.[191] These innovations allow controlled folding, unfolding, and conformational switching, leading to the creation of "smart" nanoscale devices capable of logic-based reconfiguration. Collectively, these advances have transformed DNA origami from static structural constructs into functionally programmable nanoplatforms with broad potential in nanotechnology and biomedical applications.

Compared with traditional nanocarriers such as liposomes, polymeric nanoparticles, and inorganic materials, DNA origami shows significant advantages.[192] First, DNA origami possesses a high degree of programmability and predictable architecture.[193] Benefiting from the Watson–Crick base-pairing principle, researchers can precisely control the position and pairing of each nucleotide through computer-aided design, thereby achieving customizable construction of nanoscale structures with defined morphology, size, and topology. This unprecedented level of structural accuracy allows DNA origami to be designed in various forms—such as rod-like, tubular, box-shaped, cage-like, or even dynamic architectures capable of opening, closing, or conformational switching—to meet diverse requirements for drug loading and targeted delivery. Second, DNA origami demonstrates excellent spatial addressability and functional modularity.[194] Each DNA strand within the structure can be regarded as a distinct addressable site. By extending the staple strands or introducing chemical modifications, functional entities such as proteins, peptides, chemotherapeutic drugs, nucleic acid aptamers, and siRNAs can be anchored at predetermined positions with defined copy numbers, geometric arrangements, and inter-ligand spacings. This precise spatial control provides an ideal platform for investigating multivalent interactions and constructing multifunctional or stimuli-responsive therapeutic systems. Finally, DNA origami offers notable advantages in biocompatibility and biodegradability.[195] As a nanostructure composed of natural biomacromolecules, its framework can be enzymatically degraded *in vivo* into nontoxic nucleotide byproducts, minimizing biosafety concerns. Compared with most inorganic or polymeric delivery vehicles, DNA origami achieves precise molecular delivery while offering superior intrinsic safety and greater potential for clinical translation.

Building upon these advantages, DNA origami has gradually evolved into a precisely designable and highly programmable platform for nucleic acid therapeutics, showing great potential in drug delivery, targeted recognition, and multimodal

combination therapy.[196] In programmable drug delivery, DNA origami can serve as an accurate carrier for molecular loading and controlled release. By spatially positioning nucleic acid sequences, it enables efficient transport and programmable release of therapeutic payloads. Wang *et al.* employed DNA origami technology to construct a functionalized DNA nanodevice in which siRNA was encapsulated within the inner cavity, while the chemotherapeutic drug DOX was intercalated into the DNA duplexes.[197] The incorporation of disulfide linkages into the framework allowed GSH-triggered release of siRNA under the reductive environment of tumor cells, achieving precise gene silencing of oncogenic targets and significantly suppressing cancer progression (Fig. 8D). The spatially programmable nature of DNA origami allows it to achieve multivalent recognition on the nanoscale. By precisely tuning the distance, density, and orientation of recognition motifs, researchers can systematically investigate how receptor clustering, signal transduction, and immune recognition depend on spatial organization. Zhang *et al.* constructed DNA-origami-based arrays displaying receptor-binding domains (RBDs) of SARS-CoV-2 with defined valencies and spacings, revealing how nanoscale ligand organization governs viral infection efficiency and immune activation mechanisms (Fig. 8E).[198] Hu *et al.* further designed a series of tunable multivalent aptamer-modified DNA nanostructures, in which the aptamer type, valency, binding pattern, and origami geometry were adjusted to modulate tumor-targeting selectivity.[199] Tubular origami structures were employed to deliver prodrugs into tumor cells, while sheet-like structures facilitated specific interactions between macrophages and tumor cells, thereby promoting immune clearance. These studies collectively highlight the spatial programmability of DNA origami in achieving multivalent recognition and immune modulation (Fig. 8F). In addition, DNA origami provides a modular platform for combination therapy. Xu *et al.* designed an octahedral DNA origami framework (OctDOFs) capable of co-loading siRNA, the chemotherapeutic agent DOX, and gold nanorods as photothermal agents, thereby integrating gene silencing, chemotherapy, and photothermal therapy into a single multimodal nanotherapeutic system (Fig. 8G).[200]

Overall, the emergence of nucleic acid nanostructures—such as spherical nucleic acids (SNAs) and DNA origami—has propelled nucleic acid therapeutics from sequence-level optimization toward structural-level innovation, providing new strategies for precise delivery and intelligent therapy.

3. Challenge in nucleic acid development

3.1. Delivery issues

At present, nucleic acid therapeutics are predominantly administered *via* parenteral routes, with intravenous and subcutaneous injections being the most common, while oral formulations remain in the early exploratory stage.[201]

3.1.1. Delivery barriers of intravenously administered nucleic acid therapeutics. After intravenous administration, nucleic acid therapeutics encounter multiple physiological barriers within the bloodstream.[202] First, abundant nucleases present in circulation rapidly degrade unprotected nucleic acid molecules, leading to a rapid decline in effective drug concentrations. Meanwhile, nonspecific interactions with serum proteins further reduce their bioavailability. Small nucleic acid molecules are readily cleared through glomerular filtration, while larger carrier systems are recognized and phagocytosed by the reticuloendothelial system.

The vascular endothelium—particularly specialized barriers such as the blood–brain barrier—further limits drug penetration into target tissues.[203] Only under pathological conditions (*e.g.*, tumors or inflammation) where vascular permeability is enhanced can the enhanced permeability and retention (EPR) effect facilitate drug accumulation at diseased sites. Even after traversing endothelial barriers, nucleic acid therapeutics face additional onstacles within the extracellular matrix, such as fibrosis and elevated interstitial pressure, which hinder their diffusion and effective distribution to target cells. Upon reaching target tissues, nucleic acid therapeutics must further enter target cells and, in some cases, reach specific subcellular compartments. However, the negative charges on nucleic acids result in electrostatic repulsion from the similarly negatively charged cell membrane,[204] which hinders their transmembrane transport. Cellular uptake usually relies on cationic carrier-mediated endocytosis. Once internalized, nucleic acids typically first localize to early endosomes and are subsequently trafficked to late endosomes and lysosomes, nucleic acids are initially localized to early endosomes and eventually trafficked to late endosomes or lysosomes, where the acidic environment and nuclease increase their susceptibility to degradation.[205] Efficient endosomal escape is therefore a central challenge in nucleic acid delivery, with strategies focusing on pH-responsive materials or membrane-disruptive mechanisms to enable timely release into the cytoplasm. Additionally, for nucleic acid therapeutics requiring nuclear entry, crossing the nuclear envelope is particularly challenging in non-dividing cells, often necessitating specific nuclear localization signals or carrier systems for efficient nuclear delivery.

3.1.2. Delivery barriers of subcutaneously administered nucleic acid therapeutics. Subcutaneous injection allows for sustained release and gradual absorption of nuclear acid therapeutics into the systemic circulation. However, several challenges exist during this process. Following injection, the therapeutics must diffuse through interstitial fluid and enter systemic circulation *via* capillaries or lymphatic vessels. The subcutaneous milieu is enriched with nucleases and proteases that readily degrade unprotected nucleic acid molecules during absorption, reducing their bioavailability. Furthermore, due to their negative charge and large molecular size, nucleic acids diffuse slowly, limiting their rapid entry into the bloodstream or target tissues. Additionally, the local immune system may recognize nucleic acids as exogenous molecules, thereby triggering innate immune responses.[206] Dendritic cells and macrophages, for example, can detect nucleic acid structures through pattern recognition receptors (PRRs) likely TLRs and RIG-I, which results in inflammatory cytokine release and drug

clearance *via* phagocytosis.[207] The dense extracellular matrix and low water content in subcutaneous tissues may also impede rapid distribution, causing drug retention and nonspecific binding, further delaying or even hindering systemic distribution.[208] To overcome these challenges, chemical modifications and optimization of delivery systems are commonly employed. At the molecular level, modification likely 2′-*O*-methylation and phosphorothioate modification can enhance nuclease resistance and reduce immunostimulatory effects. At the delivery system level, lipid nanoparticles (LNPs),[209] polymeric nanoparticles, and GalNAc conjugation technologies have been shown to improve subcutaneous stability and cellular uptake. Fine-tuning particle size and surface charge facilitates efficient lymphatic absorption, while incorporation of targeting ligands (*e.g.*, antibodies or aptamers) enables tissue specificity, thereby maximizing the therapeutic efficacy of subcutaneously administered nucleic acid drugs.[210]

3.1.3. Delivery barriers of intramuscularly administered nucleic acid drugs. Intramuscular (IM) administration primarily delivers drugs into skeletal muscle tissue, where the injected formulation diffuses through interstitial spaces, traverses fascial barriers, and subsequently enters capillaries or lymphatic vessels.[211] The blood flow rate within muscle tissue, injection volume, and the physicochemical properties of the drug—such as molecular size and hydrophilicity—can all influence absorption kinetics, resulting in delayed onset or fluctuating bioavailability. Moreover, nucleases and immune cells (*e.g.*, macrophages) present in muscle tissue may induce local degradation of nucleic acid therapeutics. In addition, nonspecific interactions between nucleic acids and components of the extracellular matrix can lead to drug retention at the injection site, impeding systemic circulation and reducing delivery to distal target organs. To overcome these barriers, two main strategies have been developed. First, chemical modification of nucleic acid molecules—such as alterations to the phosphate backbone or ribose moiety of siRNA and ASO—can significantly enhance nuclease resistance, thereby prolonging their residence time in muscle tissue. A representative example is the morpholino-modified ASO therapy reported by Julia Alter *et al.*, which employs a neutral phosphorodiamidate morpholino backbone to improve chemical stability and promote efficient exon skipping, restoring and sustaining dystrophin expression in skeletal muscle, thus providing a practical therapeutic option for most patients with Duchenne muscular dystrophy (DMD).[212] Second, advanced nanocarrier systems have been applied to enhance nucleic acid delivery efficiency. Most mRNA vaccines are administered *via* intramuscular injection and require protective and transport carriers to achieve effective delivery. The COVID-19 mRNA vaccines developed by Pfizer/BioNTech and Moderna both utilize lipid nanoparticles (LNPs) as delivery systems.[213] LNPs form a local "drug depot" at the injection site, facilitating efficient cellular uptake through endocytosis and promoting endosomal escape under acidic conditions, which enables the sustained release and translation of mRNA.[214]

3.1.4. Delivery barriers of intrathecal administration of nucleic acid therapeutics. Intrathecal (IT) injection delivers drugs directly into the subarachnoid space *via* lumbar puncture, allowing the formulation to enter the cerebrospinal fluid (CSF) and thereby bypass the blood–brain barrier (BBB) to act directly on the central nervous system (CNS).[215] This route has become a key administration method for the treatment of various CNS disorders, such as SMA. However, the continuous production, circulation, and reabsorption of CSF substantially shorten the residence time of therapeutics at the target site. In addition, the distribution of drugs within the CSF is often heterogeneous, resulting in steep concentration gradients and limited exposure of intracranial targets. Even when nucleic acid drugs reach the CNS, efficient translocation across the CSF–brain/spinal cord interface and subsequent uptake by neurons or glial cells remain major challenges. Furthermore, nucleases present in the CSF can degrade nucleic acids, thereby reducing transfection efficiency. To overcome these barriers, several optimization strategies have been developed. One approach is to enhance the stability and cellular uptake of nucleic acids through chemical modification. For instance, Tofersen (BIIB067), an intrathecally administered siRNA therapy developed by Novartis, targets SOD1-associated amyotrophic lateral sclerosis (ALS).[216] The molecule incorporates 2′-*O*-MOE and 2′-fluoro modifications, markedly improving its stability and cellular internalization within the CSF. Another approach focuses on improving drug distribution within the CNS. The convection-enhanced delivery (CED) technique utilizes externally applied pressure to drive the convective flow of therapeutic agents, achieving more uniform distribution throughout the CSF.[217] In addition, cationic lipid formulations and viral vectors have been explored to enhance cellular transfection efficiency following intrathecal administration. Among these, adeno-associated virus (AAV) vectors are particularly attractive owing to their intrinsic neurotropism, enabling efficient and long-term transgene expression in both neurons and glial cells. For example, Madoka Yoshimura *et al.* engineered an AAV2-MCKΔCS1 vector that demonstrated strong potential in the gene therapy of Duchenne muscular dystrophy (DMD), highlighting the capability of AAV-based systems to achieve robust and sustained gene expression in neural tissues.[218]

3.1.5. Delivery barriers of other injectable routes for nucleic acid therapeutics. In addition to the aforementioned administration routes, other injection pathways also play critical roles in the delivery of nucleic acid therapeutics, each facing distinct physiological and pharmacokinetic challenges. Intraperitoneal (IP) injection is limited primarily by the first-pass hepatic metabolism, as drugs entering the portal circulation are rapidly transported to the liver. To address this issue, researchers have designed delivery systems capable of selectively targeting specific intraperitoneal cell populations or favoring lymphatic absorption. For example, Kathryn A. Whitehead *et al.* reported an ionizable lipid nanoparticle formulation that, when administered intraperitoneally, enabled macrophage-mediated gene transfer and achieved stable and tissue-specific protein expression in the pancreas.[219] Within local injection routes, intratumoral and intravitreal administrations represent two representative strategies. Intratumoral injection is hindered by the dense extracellular matrix (ECM)

and elevated interstitial fluid pressure of tumor tissues, both of which severely restrict drug diffusion and homogeneous distribution. To overcome these barriers, smart delivery systems capable of degrading the ECM or responding to the tumor microenvironment have been developed. For instance, Robert S. Coffin reported the oncolytic immunotherapy Talimogene laherparepvec (T-VEC),[220] which selectively replicates within tumor cells and expresses granulocyte–macrophage colony-stimulating factor (GM-CSF), thereby enhancing systemic anti-tumor immunity. Intravitreal injection is a routine method for treating retinal diseases, yet rapid intraocular clearance and limited cellular transduction remain major challenges. Chemically modified antisense oligonucleotides (ASOs) and recombinant adeno-associated virus (rAAV) vectors have proven to be clinically effective strategies. For example, Fomivirsen,[221] the first FDA-approved antisense oligonucleotide drug, utilizes a phosphorothioate backbone modification to markedly enhance its stability in the vitreous and retinal tissues. For hereditary retinal disorders, Jean Bennett *et al.* employed an optimized AAV2 vector to efficiently deliver the RPE65 transgene, achieving robust transduction following either intravitreal or subretinal injection.[222]

In summary, the selection of an appropriate administration route for nucleic acid therapeutics should comprehensively consider the target organ, physicochemical properties of the molecule, and desired pharmacokinetic profile. Intravenous delivery is challenged by systemic barriers and limited cellular uptake, whereas subcutaneous and intramuscular routes are constrained by local absorption and systemic transport. In contrast, the key determinants for intrathecal and local injections lie in achieving efficient distribution, prolonged retention, and effective cellular transfection within the target region.

3.2. Stability and safety

3.2.1. *In vivo* stability and degradation mechanisms. Nucleic acid therapeutics face numerous degradation pathways *in vivo*, primarily due to the ubiquitous presence of nucleases, such as DNase I and RNase A in plasma and tissues. Unmodified oligonucleotides typically exhibit plasma half-lives ranging from minutes to a few hours, severely limiting their therapeutic window. The major degradation pathways of nucleic acid drugs include:[223] (1) Aggregation: initiated by intermolecular hydrophobic interactions, divalent ion bridging, or base pairing, which reduces solubility and leads to loss of activity. (2) Oxidation: induced by reactive oxygen species (ROS) or metal ions, resulting in base modifications or strand cleavage.[223,224] (3) Deamination: primarily at adenine and cytosine bases, where amino groups are replaced by carbonyl groups, thereby altering base-pairing properties. (4) Hydrolysis: involving cleavage of the phosphodiester backbone, with rates strongly influenced by pH, temperature, and nuclease activity. (5) Adsorption: nonspecific binding to container surfaces, serum proteins, or the surfaces of nanocarriers, which reduces biavailability. These degradation mechanisms compromise drug stability, reducingeffective concentrations and altering pharmacokinetic distribution profiles.

To improve stability, several strategies have been developed: (1) chemical modifications: phosphorothioate substitution, 2′-*O*-methylation, and incorporation of LNA structures significantly improve stability.[225] (2) Terminal capping: modifying the 3′ and 5′ ends to block nuclease recognition site.[226] (3) Conjugation with delivery systems: encapsulation in lipid nanoparticles,[227] polymers,[228] or proteins[229] can prolong circulation time, reduce degradation, and enhance bioavailability.

3.2.2. Immunogenicity and innate immune activation. Certain nucleic acid sequences can activate innate immune responses through PRRs, such as Toll-like receptors (TLR3, TLR7/8, TLR9)[230] and RIG-I-like receptors (RLRs).[231] Activation of these receptors triggers the secretion and inflammatory responses. For example, unmodified double-stranded RNA can activate TLR3, single-stranded RNA with uridine-rich sequences activates TLR7/8, and CpG-rich oligodeoxynucleotides (ODNs) can be recognized by TLR9, leading to immune activation. While such immune stimulation may have beneficial effects, such as antitumor activity, it can also cause severe adverse reactions like cytokine storms. To mitigate the immunogenicity of nucleic acid therapeutics, three major strategies are commonly employed. (1) Chemical modifications: 2′-OMe or 2′-F modification can reduce TLR7/8 activation, a method widely used in siRNA formulations.[232] (2) Sequence optimization: removing or modifying immunostimulatory motifs (*e.g.*, methylation or substitution of CpG motifs with GpC motifs) reduces TLR9-mediated immune responses.[233] (3) Delivery system optimization: encapsulation of nucleic acids in lipid nanoparticles (LNPs) limits direct PRR interactions, and combining this nucleoside modification like N1-methyl-pseudouridine reduces immunostimulatory effects. This strategy has been successfully applied in the BNT162b2 mRNA vaccine.[234]

3.2.3. Long-term safety and off-target risks. The long-term safety of nucleic acid therapeutics, particularly gene-editing technologies like CRISPR/Cas systems and RNA interference-based drugs,[235] remains a key concern. Off-target effects, where non-target genes are inadvertently modified, can lead to unpredictable biological consequences. Additionally, delivery vehicles like such as cationic lipids may cause cytotoxicity or provoke nonspecific inflammatory responses at high doses,[236] further complicating safety concerns. To systematically assess and mitigate these risks, integrated multi-omics approaches—including high-throughput sequencing and proteomic analyses—are essential for comprehensive characterization of off-target effects. Strategies such as optimizing dosing regimens, improving delivery system efficiency, and enhancing tissue specificity are critical for minimize safety concerns and expanding the therapeutic safety window. These measures will facilitate the translation of nucleic acid therapeutics from preclinical research to clinical application.

3.3. Cost and scalability

3.3.1. Raw material synthesis and quality control. The production cost of nucleic acid therapeutics is largely dictated by the synthesis stage. In chemical synthesis, the solid-phase phosphoramidite method is the industry standard for synthesizing

short oligonucleotides (<30 nt), offering high efficiency and well-established protocols. However, as sequence length increases or as chemical modifications (*e.g.*, 2′-OMe, 2′-F, pseudouridine) are introduced, synthetic efficiency declines significantly. This results in higher cycle counts, increased reagent consumption, and overall rising production costs. A notable example is the early development of commercial siRNA drugs, where raw material synthesis was a major contributor to the high production costs. On the other hand, enzymatic synthesis methods, such as *in vitro* transcription using T7 RNA polymerase,[237] are better suited for longer RNA strands (*e.g.*, mRNA), offering some cost reduction compared to chemical synthesis. Despite these advantages, enzymatic synthesis remains challenged by the high cost of modified nucleotides, inefficiencies in nucleotide incorporation efficiency, and a dependence on high-quality DNA templates, which keep overall costs high. The early production of Pfizer/BioNTech's BNT162b2, for example, faced inflated costs due to expensive modified nucleotides like N1-methylpseudouridine and low reaction yields.[238]

Following synthesis, purification and quality control processes emerge as the primary bottlenecks in cost reduction and scalability. Techniques such as high-performance liquid chromatography (HPLC), ultrafiltration, and gel electrophoresis are commonly employed to remove impurities, such as short fragments, unmodified strands, and by-products. However, these methods demand expensive equipment, high reagent consumption, and time-intensive operation, which hinder large-scale production and drive-up costs. For instance, in the production of Onpattro (patisiran), HPLC purification was a critical limiting factor that restricted capacity and significantly increased costs. Moreover, stringent quality control is required throughout the process, including LC-MS, capillary electrophoresis (CE), and qPCR to ensure sequence integrity and correct chemical modifications. For mRNA vaccines, additional tests are required to verify capping efficiency and lipid nanoparticle (LNP) encapsulation, further extending production timelines and increasing per-unit cost. While essential for ensuring safety and efficacy, these rigorous processes pose major challenges to scaling production and achieving cost reductions during commercialization.

3.3.2. Cost and scalability of delivery systems. The cost of delivery systems is another significant factor in the commercialization of nucleic acid therapeutics, especially for mRNA and siRNA drugs that require highly efficient intracellular delivery. Among available platforms, lipid nanoparticles (LNPs) are the most advanced and widely used, followed by polymeric carriers (*e.g.*, polyethyleneimine, biodegradable polyesters) and inorganic nanomaterials (*e.g.*, gold nanoparticles). LNP synthesis requires precise control over lipid composition—including ionizable lipids, cholesterol, phospholipids, and PEGylated lipids—all of which are often patent-protected and expensive. For instance, ionizable lipids like ALC-0315 and SM-102,[239] widely used in mRNA therapeutics, had substantially higher precises and limited availability during the COVID-19 vaccine rollout, significantly driving up production costs.

Large-scale LNP manufacturing typically relies on continuous-flow microfluidics, where organic and aqueous phases are mixed to produce uniform nanoparticles. While this method offers superior reproducibility, particle size control, and encapsulation efficiency compared with traditional bulk mixing, it entails high capital investment and maintenance costs, as well as stringent requirements for operational environments (*e.g.*, sterile conditions, precise temperature control). Moreover, technology transfer between production lines necessitates extensive validation batches, further inflating the fixed costs of early commercialization.

An illustrative case of the scale-up challenge occurred with of the production of COVID-19 vaccines. Despite having efficient mRNA synthesis pipelines, companies like Pfizer/BioNTech and Moderna faced significant bottlenecks in LNP encapsulation capacity. Early in BNT162b2 production, BioNTech had to rely on external partners, such as Acuitas Therapeutics and Polymun Scientific, for LNP formulation, as internal production capacity was insufficient. This resulted in delays due to equipment limitations, high demands for batch-to-batch consistency, and lengthy validation cycles.

3.3.3. Strategies for reducing costs and improving accessibility. To address the high costs and scalability bottlenecks in the commercialization of nucleic acid therapeutics, both industry and academia are actively exploring multidimensional strategies to enhance efficiency and reduce expenses. (1) Process optimization: incorporation of inorganic auxiliaries and the adoption of continuous manufacturing concepts can significantly shorten reaction times, reduce raw material waste, and minimize costs associated with equipment switching. For instance, several leading companies have integrated automated solid-phase synthesis platforms with continuous purification workflows to improve production efficiency. Notably, Bachem has introduced multi-column continuous chromatography in industrial-scale oligonucleotide production, achieving substantial reductions in solvent consumption and improved productivity.[240] In the purification stage, replacing portions of HPLC procedures with membrane filtration or implementing multi-step gradient elution strategies can maintain high purity standards while reducing solvent and consumable usage, thereby further lowering production costs. (2) Modularized manufacturing: standardizing nucleic acid synthesis platforms and delivery systems (*e.g.*, universal LNP carriers), the development of therapeutics for different indications can be achieved with minimal adjustments restricted to the target sequence. This approach not only shortens process development timelines but also significantly reduces upfront investment in clinical trials and commercial translation. For example, Moderna utilized the same LNP platform for both its COVID-19 vaccine and rare disease pipeline projects, enabling rapid switching of production lines and efficient resource utilization. (3) Raw material substitution: developing cost-effective protective groups and modified monomers with comparable performance can substantially reduce long-term raw material expenditures. For instance, replacing patented ionizable lipids with lower-cost alternatives can alleviate raw material dependency in mRNA or siRNA delivery systems and reduce cost pressures in large-scale production. (4) Globalized manufacturing: establishing GMP-compliant production facilities in regions with lower labor and raw material costs (*e.g.*, Southeast Asia or Eastern Europe) can

reduce manufacturing and logistics expenses while diversifying supply chain risks. On the other hand, developing multicenter production sites in major markets and industrial hubs enhances product accessibility and supply stability. For example, between 2021 and 2022, Pfizer and BioNTech established mRNA vaccine manufacturing bases in Belgium, Germany, and the United States, substantially improving global vaccine accessibility and providing a replicable paradigm of multicenter production for the broader nucleic acid therapeutics industry.

3.4. Long-term efficacy and adverse effect monitoring

The durability of efficacy and long-term safety are central factors in determining the clinical value of nucleic acid therapeutics. Over time, repeated dosing may lead to reduced efficacy, driven by several mechanisms. The immune system can generate specific antibodies against delivery systems, such as lipid nanoparticles or PEGylated components, leading faster drug clearance and altered tissue distribution. Additionally, target genes may evade therapeutic inhibition through mutations, production of alternative splice variants, or activation of compensatory signaling pathways.[241] Furthermore, intracellular delivery and release efficiency may decline due to factors such as saturation of RISC,[242] reduced endocytosis, or impaired endosomal escape. In addition to efficacy concers, long-term administration also raises concerns regarding chronic toxicities, including accumulation of drugs or delivery materials in organs such as the liver and kidney, persistent low-grade inflammatory responses, and potential off-target effects or genomic instability associated with gene-editing therapies.

To comprehensively evaluate these risks, a systematic and standardized long-term follow-up framework is urgently needed to enable dynamic monitoring of both efficacy and safety. Such a framework should span from baseline to multi-year assessments, incorporating not only disease-specific clinical endpoints and biomarker changes but also pharmacokinetic/pharmacodynamic data. Longitudinal monitoring should include immunological indices (*e.g.*, anti-drug antibody titers, cytokine profiles), organ function (*e.g.*, hepatic and renal function, coagulation markers), and molecular-level changes (*e.g.*, target gene expression, alternative splicing patterns, potential off-target events).[243] In parallel, integration of real-world registry studies with remote digital health monitoring can provide continuous insights into patients' quality of life and functional status, with pre-defined alert thresholds enabling timely intervention. Such a long-term monitoring framework will not only safeguard the therapeutic efficacy and safety of nucleic acid drugs across the treatment course but also provide critical evidence for the rational design and optimization of next-generation nucleic acid therapies.

4. Future perspectives and opportunities

4.1. CRISPR and gene editing therapies

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its derivative gene-editing systems (such as CRISPR–Cas9, Cas12a, and Cas13) have revolutionized nucleic acid therapeutics by enabling precise genome editing.[244] Unlike conventional strategies that rely on exogenous nucleic acids for supplementation or inhibition, CRISPR allows for direct modifications at the genomic level—including gene knockout, knock-in, and base substitution—thereby addressing pathogenic mutations at their source. Gene-editing therapies hold the potential to achieve long-lasting or even permanent therapeutic effects with a single administration and can target previously considered "undruggable". Prominent successes include the restoration of function in DMD models using Cas9, and the capacity of Cas13 to specifically target viral RNA transcripts.[245]

Despite these advances, clinical application of CRISPR faces significant challenges, particularly in delivery efficiency and safety. Currently, such as adeno-associated virus (AAV) vectors and lipid nanoparticles (LNPs) are the primary delivery systems for CRISPR components (Cas proteins and their guide RNAs).[246]

However, these approaches require a delicate balance between delivery efficiency, immunogenicity, and durability. While AAV-mediated gene editing has advanced into clinical trials for ocular and liver diseases, concerns regarding vector integration and immune response remain. Non-viral strategies, such as LNPs carrying mRNA encoding Cas proteins,[247] can mitigate integration risks and allow repeat dosing, but issues related to tissue specificity, editing efficiency, and off-target effects continue to pose significant barriers

Looking ahead, CRISPR-based therapies are expected to extend beyond monogenic disorders into complex polygenic diseases, viral infections, and cancer immunomodulation. Emerging innovations, such as high-fidelity Cas variants (*e.g.*, Cas9-HF, eSpCas9),[248] base editors (BEs), and prime editors (PEs), are improving precision and minimizing off-target risks, thereby improving editing precision and safety.[249] The integration of AI-driven guide RNA design, along with inducible systems for spatiotemporal control of gene editing, will further enhance the predictability and precision of these therapies. Together with advancements in delivery systems and monitoring technologies, CRISPR-based gene editing is poised to significantly impact precision and personalized medicine

4.2. Synthetic biology and nanotechnology

Synthetic biology offers new avenues for advancing nucleic acid therapeutics by incorporating programmable features that enhance efficacy and safety. Tools, such as synthetic promoters, toehold switches, riboswitches, CRISPRi/a systems, and base or prime editors, can be engineered to control gene expression or editing activity in response to specific signals, cell types, or timeframes.[250] This precision enables logic-gated regulation (*e.g.*, AND/NOT functions), which reduces off-target risks and systemic side effects. For example, self-amplifying RNAs (saRNAs) and circular RNAs (circRNAs) can amplify therapeutic effects and extend expression duration under limited doses.[251] In addition, "safety switches" (kill-switches) and inducible termination systems (*e.g.*, drug-inducible Cas proteins or molecular degraders) allow rapid suspension of activity in the event of adverse reactions. In terms of targeting strategies,

aptamers or peptide ligands can recognize specific receptors on cell surfaces (*e.g.*, GalNAc for hepatocyte targeting or tumor-specific aptamers),[252] thereby directing biodistribution and cellular selectivity at the earliest stages of drug delivery. This "navigation" function shifts the decision point of drug specificity to the initial delivery step, enabling integrated optimization across delivery, cellular uptake, and functional activity, which enhances therapeutic selectivity while minimizing off-target toxicity.

Nanotechnology provides a crucial role in enableing the delivery of these programmable strategies. Lipid nanoparticles (LNPs) are widely used due to their ability to efficiently encapsulate and deliver nucleic acids while ensuring effective endosomal escape. Other carriers, including polymeric and inorganic carriers, utilize stimulus-responsive designs (*e.g.*, pH, redox, or enzymatic triggers) for controlled release in specific microenvironments. DNA origami and nucleic acid nanostructures provide precise multivalent delivery,[253] enabling the co-delivery of genome-editing tools and repair templates. Biomimetic carriers, such as exosomes, enhance immune compatibility and circulation time, promoting more effective and sustained therapeutic delivery. From a translational perspective, continuous-flow microfluidic assembly and modularized quality-control systems can ensure particle size uniformity and batch-to-batch stability while reducing per-dose costs and shortening release cycles. Together, synthetic biology determines "when, where, and how" therapeutic functions are exerted, while nanotechnology ensures they are "delivered and released efficiently." The synergy of these two disciplines thus establishes an integrated pathway for nucleic acid therapeutics, spanning precise action, safety regulation, and scalable manufacturing.

4.3. Combination therapy

The integration of nucleic acid drugs with small molecules or antibodies offers synergistic therapeutic potential, particularly in enhancing pathway complementarity and reversing resistance mechanisms. For example, siRNA or ASO can downregulate key drivers or resistance factors (*e.g.*, KRAS adaptors, BCL2, ABCB1/P-gp), [254–256] thereby resensitizing tumors to targeted therapies or chemotherapy. In the immunotherapy, mRNA/siRNA modulation of immune axes such as PD-L1 and IL-12 can amplify both the depth and durability of responses to PD-1/PD-L1 inhibitors (*e.g.*, combining PD-L1 siRNA or IL-12 mRNA with anti-PD-1 therapy). Such synergies are often sequence-dependent, where nucleic acid drugs act as a "pre-conditioning" step to suppress escape or resistance pathways before chemotherapy or targeted therapy. In terms of delivery, co-encapsulation within a single carrier (ensuring co-delivery into the same cells) and separate administration (reducing formulation complexity) are viable options. Real-time decision-making can be guided by disease biomarkers and pharmacokinetic/pharmacodynamic readouts, such as target protein downregulation and inhibition of downstream signalling.

As activators of inert small-molecule prodrugs, nucleic acid drugs can function as spatiotemporally controllable "molecular switches." A representative strategy is gene- or mRNA-directed enzyme prodrug therapy (GDEPT/mRNA-DEPT), in which DNA or mRNA encoding an activating enzyme is delivered to ensure enzyme expression is restricted to the lesion site,[257] thereby converting systemically administered prodrugs into cytotoxic metabolites with high selectivity and a "bystander effect." Classical enzyme-prodrug pairs include HSV-TK/ganciclovir,[258] cytosine deaminase (CD)/5-fluorocytosine,[259] and nitroreductase/CB1954.[260] Another approach employs nucleic acid nanostructures or aptamers as "locks," which are "unlocked" upon recognition of tumor biomarkers (*e.g.*, specific receptors or miRNAs) to release embedded chemotherapeutics (such as DOX). Similarly, CRISPRa and switch-type riboswitches can be designed to induce enzyme expression under tumor-specific promoters.[261] The shared advantage of these strategies lies in restricting pharmacological activity to the lesion site, thereby markedly expanding the therapeutic window. Nonetheless, clinical translation remains hindered by several bottlenecks, including spatial co-localization and expression heterogeneity between the activation system and prodrug, immunogenicity during delivery, and challenges of manufacturing consistency across batches. For successful development, it is imperative to establish key clinical endpoints, such as tissue-specific expression levels, the ratio of prodrug to active drug *in vivo*, and long-term safety readouts (immunological and organ function). Concurrently, replicable dosing regimens and companion diagnostic frameworks must be implemented to enable genuine therapeutic synergy and toxicity reduction.

4.4. Construction of novel delivery vectors

The design of novel nucleic acid delivery vectors has evolved from focusing on single-material optimization to a more holistic, multidimensional approach that integrates material selection, structural design, functional module integration, and large-scale manufacturing. Ionizable lipids are fine-tuned for optimal loading efficiency and endosomal escape, while degradable polyesters and poly (β-amino ester) polymers help mitigate safety concerns related to accumulation.[262] Amphiphilic peptides and protein nanocages provide greater structural programmability and multivalent ligand display capabilities; while nucleic acid nanostructures such as DNA origami enable precise spatial configuration control at the nanoscale.[263]

From a structural perspective, core–shell architectures, multilamellar vesicles, or dendrimeric frameworks can simultaneously enhance stability and loading capacity. Surface modification with hydrophilic polymers or alternative hydrophobic coronas (*e.g.*, low-immunogenic PEG substitutes) prolongs circulation time and improves immune tolerance. At the functional module level, incorporation of endosomal escape elements, stimuli-responsive release mechanisms (pH, enzymatic, reductive, or light triggers), and SORT lipids enables spatiotemporally precise delivery of nucleic acid therapeutics. Finally, at the manufacturing level, continuous-flow microfluidics and modular production platforms markedly improve batch-to-batch consistency while meeting GMP requirements,

thereby laying the foundation for large-scale clinical-grade manufacturing.

Hybrid multi-carrier strategies, by integrating the advantages of distinct delivery platforms while offsetting their respective limitations, hold great promise for further expanding the therapeutic window of nucleic acid drugs. For instance, lipid-polymer nanoparticles (LPNs) employ polymeric cores to achieve enhanced mechanical stability and higher loading capacity,[264] while relying on lipid shells to ensure favorable biocompatibility and efficient endosomal escape. Lipid nanoparticles cloaked with exosomes or cellular membranes markedly improve immune evasion and confer tissue- or organ-specific targeting capabilities. Virus-like particle (VLP)–LNP composite systems enable the co-delivery or sequential release of Cas mRNA, sgRNA, and repair templates, thereby fulfilling the demands of complex genome-editing applications.[265] Moreover, combining these carriers with microneedles, injectable hydrogels, or physical triggering modalities (such as ultrasound-microbubble or magnetically responsive systems) allows for localized, efficient, and controllable drug release. It is important to note, however, that hybrid multi-component systems often encounter greater challenges in clinical translation, including formulation stability, immunogenicity, and regulatory compliance. Thus, the principle of "minimal sufficient complexity" should be followed—favoring degradable, chemically well-defined, and modular platforms with scalable manufacturing potential—while their clinical advantages must be validated through systematic pharmacodynamic and safety evaluations.

4.5. Precision-controlled drug release

Achieving precise release of nucleic acid therapeutics requires programming the "recognition–delivery–release" cascade and regulating it across both spatial and temporal dimensions. At the spatial level, receptor–ligand strategies (*e.g.*, GalNAc-mediated hepatocyte targeting, aptamer/peptide- or antibody-guided tumor selectivity)[266–268] and organ-selective lipids enable pre-selection of specific tissues and cell types.[269] At the subcellular level, optimization of ionizable lipid pKa and incorporation of endosomal escape modules allow the capture of the critical "time window" from endocytosis to cytoplasmic release. At the temporal level, stimuli-responsive mechanisms (pH, redox, enzymatic cleavage, ROS) and exogenous triggers (light, magnetic fields, ultrasound) can finely control the release rate and initiation timing. For expression-based cargos, synthetic biology tools such as riboswitches, toehold switches, and CRISPRa/i induction systems, in combination with molecular logic gates (AND/NOT), ensure that pharmacological activity is activated only when specific signals are met, thereby minimizing off-target effects and systemic toxicity. Depending on therapeutic needs, long-lasting interventions may exploit circular RNA or self-amplifying RNA (saRNA) to extend duration of action, whereas scenarios requiring intermittent stimulation can leverage microneedles or injectable hydrogels as local depots to achieve pulsatile or sequential release.

Multistage delivery and closed-loop control represent critical pathways for enhancing clinical controllability. On the one hand, coupling the three key elements of "signal sensing-conditional decision-responsive release" (*e.g.*, using disease-associated miRNAs or proteins as triggering inputs and carrier disassembly or transcriptional initiation as outputs) enables the construction of adaptive feedback release systems. On the other hand, complex therapeutic regimens can be coordinated through co-delivery within the same carrier or sequential administration in separate formulations—for example, the staged release of Cas mRNA, sgRNA, and repair templates, or the combined application of nucleic acids with small molecules.[270,271] These strategies can be dynamically calibrated by pharmacokinetic/pharmacodynamic (PK/PD) readouts, including tissue exposure levels, target inhibition rates, and cytokine profiles, to refine dosing rhythms in real time. In parallel, manufacturability and regulatory compliance must be considered. Continuous-flow microfluidics and modular CMC frameworks provide robust platforms to predefine critical quality attributes (*e.g.*, particle size and polydispersity, encapsulation efficiency, leakage rate, activation threshold, and release kinetics). Quality-by-Design (QbD) methodologies further ensure batch-to-batch consistency and reproducibility of exogenous triggers. Through such programmed, spatiotemporally integrated strategies, nucleic acid therapeutics may achieve higher therapeutic indices and more predictable clinical outcomes while maintaining safety.

Author contributions

All authors contributed to the writing and revision of the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

Data availability

No primary research results, software or code have been included, and no new data were generated or analyzed as a part of this perspective.

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