REVIEW ARTICLE

Application of nucleic acid drugs in personalized medicine

Hanmiao Yu*

School of Biomedical Sciences and Engineering, South China University of Technology, Guangzhou International Campus, Guangzhou, Guangdong, China.

Received: March 25, 2025; **accepted:** July 7, 2025.

Nucleic acid drugs represent a groundbreaking advance in therapeutic strategies, targeting genetic and molecular sites previously inaccessible to conventional small molecules and protein biologics. Therapeutic agents including antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), messenger RNAs (mRNAs), and novel gene-editing technologies such as CRISPR/Cas9 enable precise gene regulation, replacement, or editing. Recent advancements in chemical modifications and delivery systems have markedly improved the stability, cellular uptake, and therapeutic efficacy of nucleic acid drugs. Clinical applications have rapidly expanded with several FDA-approved therapies currently available including siRNA-based Patisiran and mRNA-based COVID-19 vaccines, demonstrating significant potential in treating hereditary disorders, cancers, and infectious diseases. However, challenges such as off-target effects, immunogenicity, and effective delivery to target tissues persist. Advanced delivery vehicles including lipid nanoparticles (LNPs) and exosome-based systems show promise in overcoming these limitations. This review explored recent developments in nucleic acid drug technologies, examined their diverse mechanisms of action, and discussed the current challenges and future strategies to enhance clinical application. Continued advances in genomics and biotechnology are expected to accelerate the integration of nucleic acid drugs into personalized medicine.

Keywords: nucleic acid drugs; antisense oligonucleotides; small interfering RNA; gene editing; mRNA therapeutics; drug delivery systems.

***Corresponding author:** Hanmiao Yu, School of Biomedical Sciences and Engineering, South China University of Technology, Guangzhou International Campus, Guangzhou, Guangdong 511442, China. Email: 202264640255@mail.scut.edu.cn.

Introduction

Throughout the history of modern medicine, traditional medicines have served as the primary means of disease treatment. From ancient herbal remedies to chemically synthesized modern drugs, these treatments have significantly contributed to relieving illness and saving lives. However, ongoing medical research has increasingly revealed the limitations of traditional medicines. Many diseases, particularly hereditary disorders, cancers, and chronic conditions, often involve abnormal expression of multiple genes or dysfunction of specific proteins. Traditional drugs are typically unable to precisely target these abnormalities, resulting in limited efficacy or serious side effects. Nucleic acid drugs provide promising solutions to these challenges. The Central Dogma of molecular biology, which describes genetic information flow as DNA → RNA → protein, is fundamental to understanding biological processes [1]. Beyond this classical flow, nucleic acids actively regulate gene expression within cells [2]. Furthermore, through their active or passive regulatory roles, nucleic acid molecules

can serve as therapeutic tools and drugs to intervene in disease progression [3]. Compared with traditional medications, nucleic acid drugs offer several key advantages. These drugs possess superior targeting specificity and can precisely regulate gene expression or RNA translation by sequence-specific binding to target genes or RNA, demonstrating significant potential in treating hereditary disorders and cancers [4]. Further, nucleic acid drug development is flexible and has a shorter development cycle. Advances in genomics and bioinformatics facilitate rapid computer-aided design of nucleic acid therapeutics targeting specific sequences, bypassing the lengthy compound-screening process required by traditional drug development [5]. Recent advancements in nucleic acid therapeutics, especially during the COVID-19 pandemic, have underscored their clinical importance. The rapid design, flexible adaptability, and scalability of mRNA vaccines have made them an effective global solution against infectious diseases. This success has increased interest from both the scientific community and the public, expanding the potential applications of nucleic acid technology in cancer therapy, autoimmune diseases, and other medical fields [6, 7]. The significant achievement of mRNA vaccines has spurred increased global interest in nucleic acid drug research, paving the way for innovative therapeutic approaches. In recent years, nucleic acid drugs have become a focal area in biomedical research. Their progress and future trends hold substantial significance for medical science. They offer novel approaches for precise cancer treatment, effectively targeting oncogenic gene expression, inducing apoptosis, and inhibiting tumor cell proliferation and metastasis through mechanisms like gene editing and RNA interference. Additionally, nucleic acid drugs show great potential in managing chronic conditions such as cardiovascular and neurodegenerative diseases, providing safe and effective therapeutic alternatives that improve patient quality of life and reduce healthcare burdens [8]. Given their multidisciplinary potential and extensive applicability, nucleic acid drugs are poised to play a crucial role in future drug development and precision medicine.

Development history of nucleic acid drugs

The development of nucleic acid drugs is closely linked to fundamental discoveries in molecular biology and continuous observations of biological processes. In 1953, Watson and Crick revealed the DNA double-helix structure, laying the foundation for understanding genetic information storage and transmission. In 1957, Crick introduced the Central Dogma, outlining the flow of genetic information from DNA to RNA and subsequently to protein. This concept clarified the connection between nucleic acids and protein synthesis, providing a theoretical basis for nucleic acid drug development. In the 1970s, scientists first attempted to use nucleic acid molecules to regulate gene expression. Pioneering research in this field was conducted in 1978 by Zamecnik and Stephenson at Harvard University. They developed an ASO complementary to the RNA sequence of Rous sarcoma virus (RSV), effectively inhibiting its replication [9]. This study was the first demonstration of nucleic acid molecules regulating gene expression *in vitro*, laying the groundwork for future nucleic acid therapeutics. Subsequently, antisense nucleic acid technology rapidly gained attention as a powerful gene-regulation tool, significantly advancing nucleic acid therapy research. In the late 1990s and early 2000s, key breakthroughs further propelled the development of nucleic acid drugs. In 2006, Andrew Fire and Craig Mello received the Nobel Prize in Physiology or Medicine for discovering RNA interference (RNAi) [10]. Their research revealed a natural mechanism where double-stranded RNAs (dsRNAs) specifically induced degradation of target mRNAs, enabling precise regulation of gene expression. This discovery enhanced understanding of gene regulatory mechanisms and provided a novel technological tool, spurring global advancements in nucleic acid drug research. Advances in chemical synthesis, nucleic acid modifications, and drug-

delivery technologies have substantially improved the stability, targeting specificity, and delivery efficiency of nucleic acid therapeutics. Chemical modifications to nucleic acid bases, phosphate backbones, or ribose have significantly enhanced drug stability *in vivo*, increased nuclease resistance, and prolonged half-life [11]. Additionally, innovative delivery systems such as LNPs and N-acetylgalactosamine (GalNAc)-modified vectors have improved targeted tissue delivery, increased therapeutic efficacy, and reduced adverse effects [12]. These technological advancements have greatly accelerated the integration of nucleic acid drugs into precision medicine and facilitated the clinical approval of multiple therapies.

Classification and mechanisms of nucleic acid drugs

Nucleic acid drugs can be categorized into three main types based on their mechanisms of action. The first category includes nucleic acid therapeutics that regulate protein expression by targeting nucleic acids to either enhance or suppress translation. This category encompasses ASOs, siRNAs, miRNAs, saRNAs, and the CRISPR/Cas system for precise genomic editing. The second category comprises nucleic acid drugs targeting proteins, represented primarily by aptamers that bind specifically and directly to target proteins, similarly to antibodies, thereby achieving precise molecular targeting. The third category includes nucleic acid drugs aimed at protein expression, exemplified by *in vitro* transcribed mRNA therapies. These drugs enable the synthesis of specific proteins within the body, eliciting targeted biological effects.

1. Nucleic acid drugs that target nucleic acids

(1) ASOs

ASOs are chemically synthesized, single stranded nucleic acids typically comprising 13 - 30 nucleotides, designed to complement the mRNA of a target gene. ASOs regulate gene expression through two primary mechanisms, which included that ASOs bind complementarily to target mRNA, recruiting intracellular nuclease RNase H that recognizes and binds to the ASO-mRNA hybrid duplex, leading to mRNA degradation and subsequent reduction in pathogenic protein synthesis (Figure 1a) [14]. ASOs can also disrupt translation or alter mRNA splicing by binding to specific mRNA regions (Figure 1b). By binding to the 5’ untranslated region (5’UTR), ASOs block ribosome attachment, hindering translation initiation, while binding to the 3’UTR can influence mRNA stability or its interaction with regulatory proteins, thereby affecting gene expression [15]. Furthermore, ASOs targeting aberrantly spliced mRNA sequences can correct faulty splicing processes, promoting the generation of functional proteins. Nusinersen, an ASO for treating spinal muscular atrophy (SMA), binds to the precursor mRNA of the SMN2 gene, modulating splicing to enhance the production of full-length SMN protein [16].

(2) siRNAs

siRNAs are double-stranded RNA molecules consisting of 21 – 23 nucleotides that mediate RNA interference (RNAi). Inside the cell, siRNA associates with the RNA-induced silencing complex (RISC), containing essential proteins such as Argonaute-2 (AGO2) (Figure 1c). AGO2 serves as the catalytic core of RISC, facilitating target mRNA cleavage [17]. Within RISC, the siRNA guide strand directs AGO2 to recognize and bind complementary sequences on target mRNA, which initiates AGO2-mediated mRNA degradation, thereby silencing pathogenic gene expression. The robust gene silencing capability of siRNA positions it as a promising therapeutic approach for diseases driven by pathogenic gene overexpression. siRNA has shown promise in treating pancreatic cancer by targeting the mutant KRAS gene, a critical driver of tumor progression. Delivered into cancer cells using LNPs, siRNA integrates into RISC, where the guide strand binds KRAS mRNA. The AGO2-containing RISC complex subsequently degrades KRAS mRNA, suppressing protein synthesis and inhibiting tumor proliferation, migration, invasion, and inducing apoptosis, achieving therapeutic effects [18].

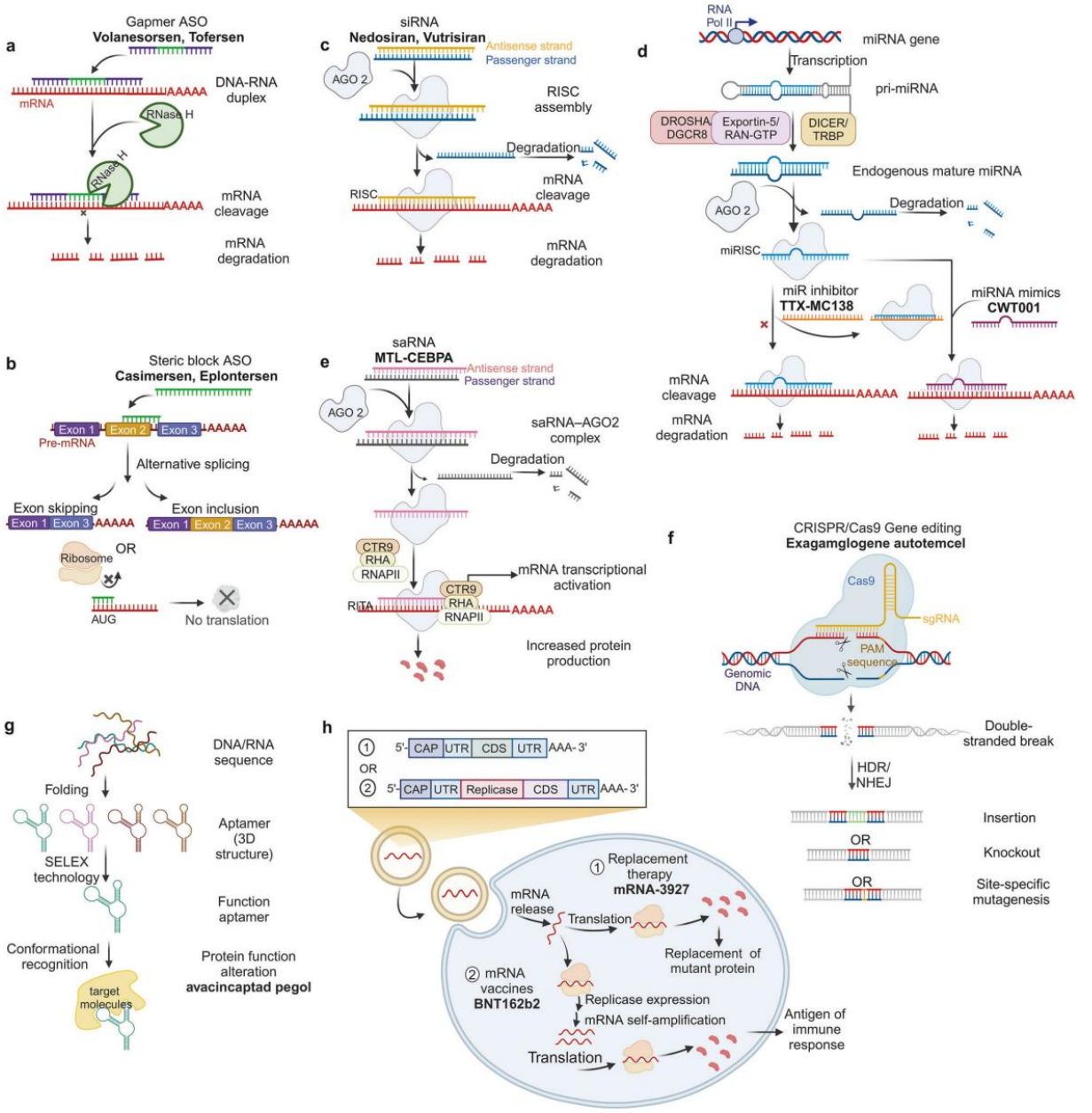

Figure 1. Classification and therapeutic mechanisms of nucleic acid drugs. **a.** Gapmer ASO forms an RNA-DNA duplex with target mRNA, causing mRNA degradation *via* RNase H. **b.** Steric-block ASO alters gene expression by promoting exon skipping/inclusion or inhibiting translation initiation by masking the AUG start codon. **c.** siRNA forms the RISC complex with AGO2, discards the passenger strand, and binds the antisense strand to the target mRNA, reducing translation. **d.** Mature miRNAs, processed by complexes such as Drosha and Dicer, form miRISC with AGO2 to regulate gene expression. miRNA inhibitors or target masking can inhibit their activity. **e.** saRNA enhances transcription by recruiting the RITA complex, composed of AGO2 and RNAP II. **f.** CRISPR uses Cas9 and sgRNA to create double-strand breaks (DSBs) in genomic DNA, repaired via homologous recombination (HDR) or non-homologous end joining (NHEJ) for gene editing. **g.** Aptamers selected through SELEX bind specific proteins by adopting unique three-dimensional structures. **h.** mRNA therapy involves exogenous mRNAs translated into proteins, applicable to protein replacement therapies and mRNA vaccines [13].

(3) miRNAs

miRNAs are endogenous non-coding RNAs, typically 19 – 25 nucleotides long, crucial for post-transcriptional gene regulation. miRNA biogenesis starts in the nucleus where RNA polymerase II transcribes primary miRNA (pri-

miRNA). The enzyme Drosha processes pri-miRNA into precursor miRNA (pre-miRNA) characterized by a stem-loop structure. Exportin-5 transports pre-miRNAs to the cytoplasm where Dicer, an RNase III enzyme, converts them into mature miRNAs. Mature miRNAs integrate into RISC, guiding it to bind complementary sequences in the 3'UTR of target mRNAs, which inhibits translation or promotes mRNA degradation, enabling precise post-transcriptional gene regulation (Figure 1d). Victor Ambros and Gary Ruvkun received the 2024 Nobel Prize in Physiology or Medicine for their pioneering discovery of miRNAs and their role in post-transcriptional gene regulation. Research in *Caenorhabditis elegans* showed that miRNAs such as lin-4 and let-7 regulate developmental timing by binding to complementary sequences in the 3'UTR of target mRNAs, inhibiting their translation [19]. This discovery introduced a new paradigm in molecular biology, revealing RNA as a regulatory molecule beyond its classical messenger or structural roles and elucidates the mechanism by which miRNAs form the RISC complex and repress mRNA translation [20]. The evolutionary conservation of this mechanism emphasizes the fundamental regulatory role of miRNAs, challenging the traditional protein-centric view of cellular regulation [20].

(4) Small activating RNAs (saRNAs)

saRNAs are double-stranded RNA molecules of 21 – 27 nucleotides that transcriptionally enhance gene expression. Unlike siRNAs or miRNAs that suppress gene expression, saRNAs activate genes by targeting DNA promoter regions. This phenomenon, termed RNA activation (RNAa), is mediated by sequence-specific interactions between saRNAs and complementary sequences in target gene promoters [21]. saRNA-mediated gene activation involves recruiting the RNA-induced transcriptional activation (RITA) complex, comprising Argonaute proteins (AGO2) and transcriptional co-activators. saRNAs bind to promoter-associated RNAs (paRNAs) or directly to promoter DNA, facilitating epigenetic changes that promote transcription. These modifications include increased histone acetylation and CpG island demethylation, establishing an open chromatin structure favorable for gene activation (Figure 1e). One promising application of saRNAs is cancer therapy, where they can reactivate epigenetically silenced tumor suppressor genes. saRNAs targeting p21 have shown anticancer effects, inducing cell cycle arrest and apoptosis across various cancer cell lines. Additionally, saRNAs can enhance expression of therapeutic genes involved in tissue regeneration and immune modulation. Efficient delivery of saRNAs is critical for therapeutic success. Advanced delivery methods such as LNPs, viral vectors, and chemical modifications like GalNAc conjugation have been developed to improve the stability and cellular uptake of saRNAs *in vivo*.

(5) CRISPR/Cas9 system

The CRISPR/Cas9 system, originally derived from bacteria and archaea as an adaptive immune defense, allows precise genome editing by targeting specific DNA sequences [22]. In this system, the Cas9 protein specifically recognizes target DNA sequences guided by single-guide RNA (sgRNA). sgRNA, formed by combining CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA), binds complementary regions flanking the protospacer adjacent motif (PAM) sequence. The Cas9 protein introduces DSBs in target DNA *via* its RuvC and HNH catalytic domains. Cells subsequently repair these breaks through either NHEJ or HDR, enabling targeted gene editing (Figure 1f). CRISPR/Cas9 is widely recognized for its potential in nucleic acid drug development, particularly gene therapy. By designing specific sgRNAs, CRISPR/Cas9 can target and cleave disease-causing genes such as those mutated in β-thalassemia and Duchenne muscular dystrophy [23]. Additionally, this system can regulate gene expression by disrupting promoter or enhancer regions or restoring gene function through HDR-mediated insertion of therapeutic genes. Recently, *in vivo* nucleic acid drug development based on CRISPR technology has entered clinical trials, demonstrating substantial therapeutic promise [24].

2. Nucleic acid drugs that target proteins

Aptamers are small nucleic acid or peptide molecules that bind with high affinity and specificity to target molecules such as proteins, small molecules, and cells due to their unique three-dimensional structures. These molecules are selected *in vitro* through a process called systematic evolution of ligands by exponential enrichment (SELEX) (Figure 1g). Aptamers regulate cell functions by blocking protein-protein interactions, inhibiting enzyme activity, or disrupting signaling pathways. As an emerging class of nucleic acid drugs, aptamers show significant potential for treating cancer, inflammation, viral infections, and various other diseases. Pegaptanib, the first FDA-approved aptamer drug, targets vascular endothelial growth factor (VEGF) to treat age-related macular degeneration by inhibiting abnormal angiogenesis [25]. Additionally, aptamers targeting cancer-related markers such as prostate-specific membrane antigen (PSMA) have shown considerable advantages in cancer diagnosis and targeted drug delivery [26].

3. Nucleic acid drugs that express proteins

Nucleic acid drugs expressing proteins represent a novel therapeutic strategy designed to generate therapeutic proteins directly in patients by delivering nucleic acid molecules encoding specific proteins such as mRNA or plasmid DNA (pDNA). This method bypasses the complex manufacturing processes required for traditional protein drugs and utilizes the patient's own cellular machinery to rapidly produce the therapeutic protein. Upon entering cells *via* a delivery system, nucleic acid molecules such as mRNA or pDNA are recognized by intracellular translation mechanisms and translated into target proteins [27]. For mRNA-based drugs, translation occurs directly in the cytoplasm. In contrast, pDNA needs to first enter the nucleus, be transcribed into mRNA by the host's transcription machinery, and then be translated in the cytoplasm (Figure 1h). Significant progress has been made in vaccine development using protein-expressing nucleic acid drugs. COVID-19 mRNA vaccines such as BNT162b2 and mRNA-1273 induce immune responses by delivering mRNA encoding spike proteins, resulting in antigenic protein expression in host cells [28].

The current clinical application landscape of nucleic acid drugs

1. Overview of approved drugs

Currently, more than ten nucleic acid drugs have received regulatory approval. This review focused primarily on several nucleic acid therapeutics introduced between 2023 and 2024.

(1) Tofersen

Biogen developed Tofersen (Qalsody™), an ASO, for treating amyotrophic lateral sclerosis (ALS). Tofersen received approval in the United States on April 25, 2023, to treat ALS in adults harboring mutations in the superoxide dismutase 1 (SOD1) gene. Miller *et al*. assessed the safety and efficacy of Tofersen in adults with ALS due to SOD1 mutations, focusing primarily on safety and pharmacokinetics. The results showed that intrathecal administration of Tofersen reduced cerebrospinal fluid (CSF) SOD1 protein levels within 12 weeks. Some participants receiving Tofersen developed endocytosis of CSF. Most observed adverse events were related to lumbar puncture procedures. A subsequent phase 3 randomized, double-blind, placebo-controlled trial and its long-term extension study continue to evaluate the safety and efficacy of Tofersen [29]. In the phase III VALOR study, although Tofersen did not meet its primary endpoint, it demonstrated positive effects in biomarker analyses. Compared with placebo, the Tofersen treatment group exhibited significantly reduced plasma neurofilament light chain (NFL) levels and CSF SOD1 protein levels [30]. These results suggest that Tofersen may delay the progression of SOD1-ALS and possesses a favorable safety profile. China has one of the largest ALS patient populations globally with research indicating over 40,000 ALS patients including more than 1,200 patients carrying SOD1 mutations. On October 8, 2024, the National Medical Products

Administration (NMPA) of China officially approved the import of Tofersen injection developed by Biogen Inc. Tofersen, as China's first disease-modifying treatment for SOD1-ALS, represents a significant advancement in rare disease treatment. This milestone provides new hope for Chinese ALS patients and highlights China's research and innovation capacity in rare disease therapeutics.

(2) Nedosiran

Nadosilan, developed by Novo Nordisk and Dicerna, received FDA approval on September 29, 2023. It is indicated for reducing urinary oxalate levels in patients aged 9 years and older with type 1 primary hyperoxaluria (PH1) who have relatively preserved renal function. Nedosiran represents the first RNA interference (RNAi) therapy and the second FDA-approved drug worldwide for PH1 treatment. PH is a rare genetic disorder characterized by excessive oxalate production, which leads to calcium oxalate kidney stone formation, potentially progressing to end-stage renal failure. PH is classified into three types as PH1, PH2, and PH3. PH1 arises from mutations in the AGXT gene, which encodes alanine-glyoxylate aminotransferase (AGT). Mutations in genes encoding glyoxylate reductase (GR) and 4-hydroxy-2-oxoglucuronate aldolase (HOGA) proteins cause PH2 and PH3, respectively. These mutations cause autosomal recessive disorders of glyoxylate metabolism, impairing hepatic glyoxylate detoxification and resulting in excessive oxalate production *via* lactate dehydrogenase A (LDHA). The liver-produced oxalate is excreted through the kidneys, leading to calcium oxalate accumulation and renal damage. Nedosiran is a siRNA therapeutic targeting LDHA, a crucial enzyme in oxalate overproduction, which specifically enters hepatocytes *via* clathrin-mediated endocytosis, facilitated by GalNAc modifications that bind specifically to hepatocyte asialoglycoprotein receptors (ASGPR). After entering hepatocytes, Nedosiran binds specifically to LDHA mRNA. The RNA-induced silencing complex (RISC) cleaves LDHA mRNA, thus suppressing LDHA expression and reducing oxalate production, effectively treating PH1. The PHYOX2 study (NCT03847909) is a randomized, double-blind clinical trial evaluating Nedosiran compared with placebo in PH1 patients aged 6 years and older. Participants receive monthly doses of Nedosiran or placebo. The primary efficacy endpoint is the percentage change from baseline in 24-hour urinary oxalate excretion, measured by the area under the curve (AUC) from days 90 to 180. The study reported that the Nedosiran group had an average 24-hour urinary oxalate (Uox) AUC of −3,486 compared to 1,490 for the placebo group. At 90, 120, 150, and 180 days, the least squares (LS) mean percentage change in urinary oxalate excretion was −37% in the Nedosiran group compared with 12% in the placebo group.

(3) Exagamglogene autotemcel (exa-cel)

On November 16, 2023, Vertex Pharmaceuticals and CRISPR Therapeutics announced that the UK Medicines and Healthcare products Regulatory Agency (MHRA) had granted conditional marketing authorization for the CRISPR/Cas9 gene-editing therapy CASGEVY™ (exagamglogene autotemcel, exa-cel, or ctx001) for treating sickle cell disease (SCD) and transfusion-dependent β-thalassemia (TDT). CASGEVY™ represents the world's first approved CRISPR/Cas9 gene-editing drug [31]. Exa-cel is an innovative cell-based gene therapy addressing the genetic basis of severe hemoglobinopathies such as SCD and TDT. These diseases result from mutations in the β-globin (HBB) gene, causing defective or insufficient hemoglobin production. In SCD, a point mutation in the HBB gene replaces glutamine with valine at position six, causing hemoglobin polymerization under low oxygen conditions, which leads to red blood cell sickling, vascular blockage, and hemolysis. Similarly, TDT involves significantly reduced or absent β-globin synthesis, causing severe anemia that requires lifelong transfusions. Increasing fetal hemoglobin (HbF) expression can alleviate these conditions. HbF, naturally present during fetal development, remains unaffected by the β-globin mutation. The transcription factor BCL11A suppresses γ-globin gene expression postnatally, reducing HbF

levels. Individuals with BCL11A mutations maintain high HbF levels into adulthood, significantly mitigating symptoms of SCD and TDT. Exa-cel employs CRISPR-Cas9 technology to edit hematopoietic stem and progenitor cells (HSPCs) by targeting a red blood cell-specific enhancer of the BCL11A gene. Initially, autologous CD34+ HSPCs are collected from the patient. Cas9 protein and single-guide RNA (sgRNA) targeting the BCL11A enhancer region are introduced into these cells *via* electroporation. The CRISPR-Cas9 complex creates DSBs at the GATA1-binding site in the enhancer region. NHEJ repairs these breaks, causing insertions or deletions (indels) that disrupt enhancer function. Reduced BCL11A expression in red blood cells reactivates γ-globin expression, elevating HbF levels. The modified HSPCs are expanded *ex vivo* and reinfused into the patient, providing a durable source of red blood cells producing elevated HbF. Exa-cel thus mitigates the pathological symptoms of SCD and TDT, offering potentially curative outcomes. Preclinical and clinical studies strongly support the efficacy and safety of this therapeutic approach. Research has demonstrated the feasibility of CRISPR/Cas9-based gene editing for treating genetic diseases such as TDT and SCD. Patients receiving CTX001 (exa-cel) experienced significant, rapid, and sustained increases in HbF within 12 months with gene-edited cells constituting more than 99% of circulating blood cells. Long-term follow-up showed persistent gene editing implantation, high HbF expression, and elimination of vaso-occlusive crises and transfusion dependence. These findings indicate CRISPR/Cas9 gene editing as a viable treatment for genetic disorders [32].

(4) Avacincaptad pegol

Izervay (Avacincaptad pegol), an aptamer-based drug, recently received approval for treating geographic atrophy (GA) secondary to dry age-related macular degeneration (AMD). GA involves progressive retinal degeneration, causing irreversible vision loss. Avacincaptad pegol functions as a complement C5 inhibitor, targeting a critical component of the complement cascade implicated in AMD pathophysiology. By inhibiting C5, it reduces formation of the membrane attack complex (C5b-9), thereby decreasing inflammation and retinal cell death. Clinical trials demonstrated that Avacincaptad pegol slowed GA progression, providing an essential therapeutic advancement for this unmet medical need [33].

(5) mRNA-1345

Respiratory syncytial virus (RSV) is a highly infectious pathogen that causes respiratory infections in individuals of all ages, particularly infants under 5 years of age. Millions of children are hospitalized annually due to RSV infections, most frequently infants younger than 6 months. Individuals previously infected with RSV remain susceptible to reinfection, particularly infants, immunocompromised patients, and adults over 65. mRNA-1345 is an mRNA-based nucleic acid therapeutic designed to prevent RSV infection. This innovative vaccine utilizes LNP technology for effective delivery. The vaccine's mRNA encodes RSV antigenic proteins, which, upon translation in host cells, elicit robust immune responses, thereby providing protection against RSV. This method leverages the advantages of mRNA therapeutics including rapid development timelines and high immunogenic potency. Currently, specific treatments for RSV infection are lacking. Vaccination has thus become crucial for preventing severe RSV-related illness and reducing mortality. On May 31, 2024, the U.S. FDA approved Moderna's mRNA-1345 (mRESVIA) to prevent RSV-associated lower respiratory tract disease in adults over 60 years, which marked the first RSV mRNA vaccine and Moderna's second major authorization following its COVID-19 mRNA vaccine. The approval was based on favorable outcomes from pivotal clinical trials, achieving two primary efficacy endpoints. Vaccine efficacy against RSV-related lower respiratory tract disease was 83.7% defined by two or more symptoms and 82.4% defined by three or more symptoms [34].

2. Clinical application of nucleic acid drugs

Nucleic acid drugs theoretically enable treatment

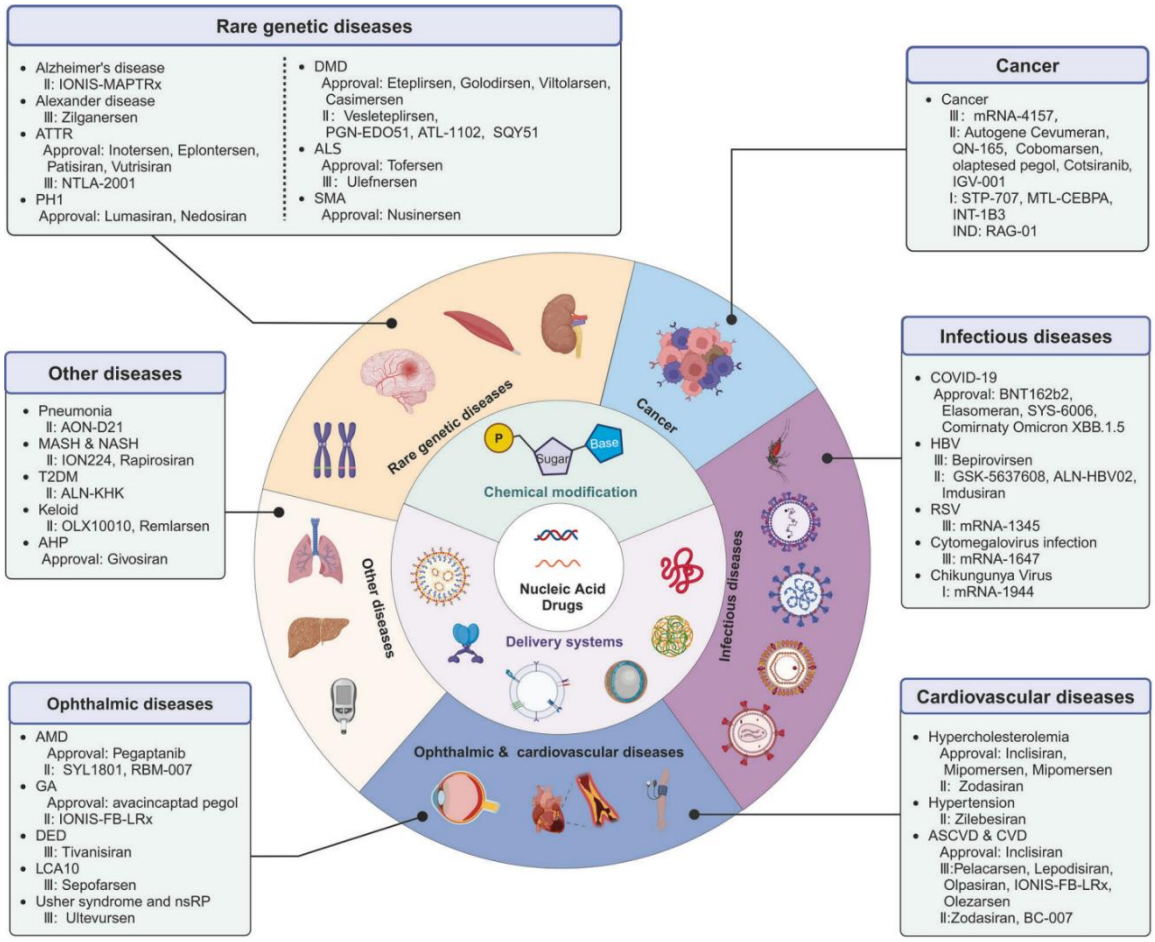

Figure 2. Clinical application of nucleic acid drugs [13].

for any disease by selecting appropriate nucleotide sequences targeting specific genes. Their distinctive physicochemical and biological characteristics offer sustained or potentially curative effects, differing from conventional therapeutics. Many nucleic acid drugs have transitioned from research to clinical application, receiving approval for clinical trials (Figure 2).

(1) Rare genetic diseases

Duchenne muscular dystrophy (DMD) is a genetic disorder caused by mutations leading to dystrophin deficiency. Exon-skipping therapy utilizes ASOs to restore partial gene function in DMD patients. In 2016, the FDA approved Eteplirsen (Exondys 51) as the first ASO-based drug for DMD. Studies demonstrated that weekly intravenous injections of Eteplirsen significantly increased dystrophin expression. Subsequently, Golodirsen (Vyondys 53) and Viltolarsen (Viltelso) were approved to induce exon 53 skipping [35].

(2) Cancer

Preparation of mRNA-4157 involves whole-exome sequencing of tumor and normal patient tissues followed by bioinformatic analysis to identify highly immunogenic tumor-specific neoantigens. These selected neoantigens are encoded into mRNA sequences formulated as personalized vaccines. Post-vaccination, mRNA-4157 is translated into neoantigen proteins *in vivo* presented by antigen-presenting cells (APCs) and stimulates CD8+ and CD4+ T cell immune responses. This process facilitates tumor cell destruction and prevents recurrence. Merck &

Co. and Moderna have initiated phase III randomized clinical trials (INTerpath-001, NCT05933577; INTerpath-002, NCT06077760) to evaluate mRNA-4157 (V940) combined with KEYTRUDA as adjuvant therapy for patients with high-risk melanoma (stage IIB–IV) and surgically resected non-small cell lung cancer [36].

(3) Infectious diseases

Following the global COVID-19 outbreak in 2019, mRNA vaccine development rapidly progressed, playing a key role in pandemic management. The short development timelines and efficacy of mRNA vaccines have been validated in both animal and clinical studies. A large randomized, placebo-controlled clinical trial conducted by Bettini *et al*. demonstrated that a two-dose regimen of Tozinameran (BNT162b2) provided 95% protection against COVID-19 in individuals aged 16 years and older. Fatigue and headache were the most common adverse reactions during vaccination [37]. Compared to the initial two doses, a third BNT162b2 dose demonstrated an efficacy of 95.3% [28]. The FDA approved BNT162b2 in 2020.

An analysis of the challenges confronting nucleic acid drug development

1. Stability concerns

Nucleic acid drugs circulate in the bloodstream for prolonged periods. Although blood nuclease concentrations are relatively low, continuous exposure makes these drugs susceptible to degradation. Additionally, the negative charge of nucleic acid molecules facilitates binding to plasma proteins, further accelerating degradation. This process significantly shortens their half-life, necessitating higher dosages [38]. Upon entering target cells, nucleic acid drugs rapidly undergo degradation by intracellular nucleases. The cytoplasm and nucleus contain abundant endonucleases and exonucleases that degrade nucleic acids, limiting their intracellular concentration. This issue is particularly pronounced for therapeutics such as siRNA and mRNA as their inherently low delivery efficiency exacerbates stability and efficacy challenges within cells [39].

2. Immunogenicity concerns

Nucleic acid sequences may mimic pathogen-associated molecular patterns (PAMPs), activating innate immune receptors such as Toll-like receptors (TLRs). Research demonstrated that unmethylated CpG motifs in nucleic acids were recognized by TLR9, triggering immune responses. Additionally, impurities from nucleic acid drug production and delivery system components increased immunogenicity, raising the risk of adverse immune reactions [40].

Strategies for Overcoming the Challenges

1. Chemical modification of nucleic acid drugs

Researchers have employed various chemical modifications to nucleic acid drugs, enhancing their pharmacological properties and resistance to enzymatic degradation. These modifications include base modifications, phosphate group modifications, ribose modifications, ribose-phosphate backbone alterations, and combinations of these strategies.

(1) Phosphate group modification

Phosphate groups are typically modified at non-bridging oxygen atoms. Substituting these oxygens with sulfur atoms produces thiophosphoric acid (PS-DNA/RNA), representing a first-generation chemical modification. This modification increases resistance to enzymatic degradation and facilitates straightforward, solid-phase synthesis, becoming a widely used method. However, thiol modifications have limitations including weaker binding affinity to target sequences. Additionally, high thiophosphate content may cause adverse effects such as cytotoxicity and immune stimulation. Therefore, both the position and quantity of thiophosphate bonds are critical for delivery efficiency. Other phosphate modifications involve replacing non-bridging or bridging oxygen atoms with amino or boroalkyl groups. The phosphate group itself can also be

substituted entirely by amido, aminoxyl, alkoxy, or triazolyl groups. These alternatives enhance anti-enzymatic stability of nucleic acid structures but are less commonly used compared to thiol modifications [41].

(2) Base modification

Base modifications primarily involve substituent alterations or base substitutions, typically at the 5-position of pyrimidines and the 8-position of purines. Commonly used base modifications include pseudouridine, 2-thiouridine, N1-methylpseudouridine, 5-methyluridine, 5-methoxyuridine, N6-methyladenosine, and 5-methylcytidine. Replacing uracil with pseudouridine is one of the most frequent base modifications.

(3) Ribose modification

Ribose modifications mainly include two types. The first involves introducing groups of varying sizes and polarities at the 2'-position such as 2'-methoxy, 2'-methoxyethoxy, and 2'-deoxy-2'-fluoro modifications [42]. The second involves simultaneous modifications at the 2'-position and other ribose sites, exemplified by locked nucleic acids (LNAs) and phosphorodiamidate morpholino oligomers (PMOs). The modifications 2'-methoxy, 2'-methoxyethoxy, and 2'-deoxy-2'-fluoro constitute second-generation antisense nucleic acid modifications. They exhibit considerable similarity and enhance the affinity between nucleic acid drugs and target sequences. However, these modifications are not recognized by RNase H, thus blocking target sequence function through steric hindrance upon binding. LNA is a conformationally restrictive modification adopting a C3'-endo conformation, maintaining high target affinity and enzymatic resistance even with short sequences. However, short-sequence LNAs pose increased off-target and toxicity risks. Thus, alternative modifications like unlocked nucleic acids, restricted ethyl-bridged nucleic acids, tricyclic DNA, and ethylene glycol nucleic acids are frequently used. Combinations of these ribose modifications with thiophosphate modifications achieve superior therapeutic outcomes. The electrically neutral structure conferred by PMOs and peptide nucleic acid modifications significantly improves nucleic acid drug stability and affinity. PMOs demonstrate good water solubility and are widely utilized with some PMO-modified siRNAs and miRNAs currently in clinical trials [43].

2. Innovations in delivery technologies

Although chemical modifications enhance nucleic acid drug stability, affinity, and delivery efficiency, their large size, negative charge, and hydrophilicity hinder cellular permeability, limiting intracellular efficacy. Therefore, selecting suitable delivery vehicles and developing efficient delivery technologies are crucial for successful nucleic acid drug therapies. An optimal nucleic acid drug delivery system must fulfill five criteria including excellent biocompatibility without immune responses, prolonged blood circulation and nuclease resistance, effective targeting and accumulation at lesion sites, high cellular uptake efficiency, and rapid and effective endosomal escape after cellular entry. In the 1980s, viral vectors were first employed to deliver exogenous genes into specific cells *in vivo*. However, immune responses and safety concerns limited their effectiveness. During the 1990s, researchers utilized liposomes, polymers, and metal nanoparticles as carriers, significantly enhancing nucleic acid drug stability and biocompatibility.

(1) Viral vectors

Viral vectors represent key tools for nucleic acid drug delivery due to their inherent gene-transduction capabilities and high cellular infection efficiency. Frequently used viral vectors include adeno-associated virus (AAV), adenovirus (Ad), retrovirus (RV), and lentivirus (LV). These vectors undergo genetic engineering to remove pathogenic genes and insert therapeutic nucleic acid sequences, enabling efficient gene delivery to target cells [44, 45]. Although viral vectors demonstrate excellent efficiency and reliability for nucleic acid delivery, they still face challenges such as immune responses, complex manufacturing processes, and limited delivery dosages. Future research may enhance delivery

efficiency and safety by optimizing viral capsids, developing engineered viral vectors, and integrating chemical delivery methods.

(2) LNPs

LNPs are self-assembled nanostructures approximately 100 nm in diameter, which bind electrostatically with negatively charged nucleic acids. LNP typically comprise four components including ionizable lipids, helper lipids such as DSPC, cholesterol, and polyethylene glycol (PEG)-modified lipids. Ionizable lipids remain neutral at physiological pH to minimize toxicity. Under acidic conditions such as in endosomes, these lipids become positively charged, facilitating interaction with endosomal membranes, promoting endosomal escape, and enhancing nucleic acid drug bioavailability. The ionizable lipid DLin-MC3-DMA is utilized in Onpattro® (Patisiran), the first approved siRNA therapeutic for hereditary transthyretin amyloidosis [46]. LNP technology has significantly advanced mRNA delivery, notably in COVID-19 vaccines such as BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna). These vaccines use LNPs to encapsulate and deliver mRNA encoding the SARS-CoV-2 spike protein to immune cells, stimulating robust immune responses [28].

(3) Exosomes

Exosomes are membranous vesicles with diameters of approximately 40 - 160 nm, released when multivesicular bodies fuse with the plasma membrane. As natural nanocarriers, exosomes can load various therapeutics including small molecules, nucleic acids, recombinant proteins, and CRISPR/Cas9. Exosomes possess unique ligands or adhesion molecules on their membranes, facilitating drug release through membrane interactions or cellular uptake [47]. Therefore, exosomes represent effective intracellular delivery systems for nucleic acid-based therapeutics. Exosomes offer distinct advantages as endogenous nanoparticles including low immunogenicity, excellent biocompatibility, safety, and enhanced capability to cross biological barriers such as the blood-brain and gastrointestinal barriers. Extensive studies have investigated exosomes for nucleic acid delivery. With advances in CRISPR technology, researchers have explored exosomes to deliver CRISPR/Cas9 constructs for cancer therapy through gene editing. Kim *et al*. demonstrated that exosomes derived from ovarian cancer cells could deliver CRISPR/Cas9 plasmids into SKOV3 xenograft mouse models, effectively inhibiting poly (ADP-ribose) polymerase 1 (PARP1) expression. Intratumoral injections maintained stable tumor volumes over 20 days, while tumors in the control group continued to grow [48]. Numerous additional studies have shown exosomes effectively deliver nucleic acid therapeutics including ASOs, siRNAs, miRNAs, and mRNAs with promising anti-tumor therapeutic effects [49].

(4) Inorganic nanoparticles (INPs)

Inorganic nanoparticles (INPs) are delivery systems composed of materials such as gold, silver, calcium phosphate, graphene oxide (GO), quantum dots, and magnetic nanomaterials like iron oxide. INPs are valued for their unique electrical and optical properties, biocompatibility, and low cytotoxicity. Gold nanoparticles (AuNPs) have versatile surfaces that facilitate direct binding with nucleic acids. Son *et al*. developed a pH-sensitive siRNA-AuNP delivery system using the i-motif secondary structure, targeting PLK1, a gene essential for chromosome stabilization and mitosis, to induce apoptosis. The changes in pH triggered conformational shifts in nucleic acids, inducing aggregation of siRNA-AuNP complexes and facilitating endosomal escape and siRNA release [50]. AuNPs offer excellent stability and precise control over nucleic acid conjugation through surface modification. However, high cost and potential toxicity associated with long-term accumulation in tissues remain significant challenges. GO, a carbon allotrope, exhibits unique optical, thermal, and electrical properties. Noncovalent π-π stacking interactions in GO enhance drug loading capacity and allow controlled release. Kim *et al*. designed a stimuli-responsive nucleic acid delivery vehicle composed of polyethylene glycol (PEG),

polyethyleneimine (PEI), and GO. Upon absorbing near-infrared radiation, GO nanoparticles generated localized heat, causing endosomal rupture and controlled release of cargo [51]. Additionally, GO has high loading capacity and can form nanocomplexes with PEI and sodium poly-4-styrenesulfonate to simultaneously deliver miRNA drugs and the anticancer drug Adriamycin [52]. GO's strengths include high loading efficiency and responsiveness to external stimuli. Nevertheless, challenges in large-scale production and long-term biological safety remain as interactions between GO and biological systems require further investigation. Inorganic nanoparticles present promising opportunities for nucleic acid drug delivery. However, understanding and addressing each nanomaterial's unique advantages and limitations are crucial for successful clinical application.

Conclusions

Nucleic acid drugs have significantly advanced from initial research stages to clinical application. Progress in understanding nucleic acid mechanisms, alongside improved synthesis, modification, and delivery technologies has led to notable achievements. The approval of multiple nucleic acid drugs and extensive clinical trials underscore this field's importance and potential. However, unresolved issues and controversies remain including variations in drug efficacy and side effects associated with different delivery methods, stimulating ongoing debate among researchers. In the next 5 - 10 years, key technological breakthroughs in nucleic acid drugs are anticipated. Integrating artificial intelligence (AI) and machine learning into nucleic acid sequence design and target screening will revolutionize drug discovery, enhancing precision and efficiency. Hybrid delivery systems combining advantages of various carriers will emerge, addressing current limitations and ensuring stable, efficient nucleic acid drug delivery to targeted tissues. Clinically, nucleic acid therapeutics will increasingly impact treatments for rare diseases, cancer, and infectious diseases. For rare diseases, advanced gene-editing technologies like CRISPR/Cas9 will offer precise genetic corrections with reduced off-target effects, potentially providing cures. In cancer treatment, personalized nucleic acid vaccines tailored to individual tumor profiles will enhance immunotherapy specificity and improve therapeutic outcomes. Regarding infectious diseases, rapid development and deployment of nucleic acid-based vaccines, particularly mRNA vaccines, will facilitate more effective preventive and therapeutic strategies. However, accomplishing these objectives requires ongoing interdisciplinary collaboration among biologists, chemists, material scientists, and AI specialists. Additionally, ethical considerations and public acceptance must be adequately addressed to ensure sustainable development of nucleic acid therapeutics. By overcoming these challenges, nucleic acid drugs are poised to become a cornerstone of precision medicine, ushering in a new era of healthcare.

References

1. Yang K. 2024. Two versions of the central dogma of molecular biology exist. Chin J Biochem Mol Biol. 40(4):25653.
2. Zhang S, Zhou Y, Wang Y, Wang Z, Xiao Q, Zhang Y, *et al*. 2021. The mechanistic, diagnostic and therapeutic novel nucleic acids for hepatocellular carcinoma. Brief Bioinform. 22(2):1860–1883.
3. He J. 2017. Research progress of nucleic acid drugs. Int J Pharm Res. 44(11):1028–1051.
4. Dowdy SF. 2017. Overcoming cellular barriers for RNA therapeutics. Nat Biotechnol. 35(3):222–229.
5. Kole R, Krainer AR, Altman S. 2012. RNA therapeutics: Beyond RNA interference and antisense oligonucleotides. Nat Rev Drug Discov. 11(2):125–140.
6. Kowalski PS, Rudra A, Miao L, Anderson DG. 2019. Delivering the messenger: Advances in technologies for therapeutic mRNA delivery. Mol Ther. 27(4):710–728.
7. Hou X, Zaks T, Langer R, Dong Y. 2021. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 6(12):1078–1094.
8. Wang F, Zuroske T, Watts JK. 2020. RNA therapeutics on the rise. Nat Rev Drug Discov. 19(7):441-442.
9. Zamecnik PC, Stephenson ML. 1978. Inhibition of Rous sarcoma virus replication by a specific oligodeoxynucleotide. Proc Natl Acad Sci. USA. 75(1):280–284.

10. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 391(6669):806–811.
11. Crooke ST, Baker BF, Crooke RM, Liang XH. 2021. Antisense technology: An overview and prospectus. Nat Rev Drug Discov. 20(6):427–453.
12. Zatsepin TS, Kotelevtsev YV, Koteliansky V. 2016. Lipid nanoparticles for targeted siRNA delivery. Int J Nanomedicine. 11:3077–3086.
13. Sun X, Setrerrahmane S, Li C, Hu J, Xu H. 2024. Nucleic acid drugs: Recent progress and future perspectives. Signal Transduct Target Ther. 9(1):316.
14. Crooke ST, Wang S, Vickers TA, Shen W, Liang XH. 2017. Cellular uptake and trafficking of antisense oligonucleotides. Nat Biotechnol. 35(3):230–237.
15. Rinaldi C, Wood MJA. 2018. Antisense oligonucleotides: The next frontier for treatment of neurological disorders. Nat Rev Neurol. 14(1):9–21.
16. Hua Y, Sahashi K, Hung G, Rigo F, Passini MA, Bennett CF, *et al*. 2010. Antisense correction of SMN2 splicing rescues necrosis in SMA mice. Genes Dev. 24(15):1634–1644.
17. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference. Nature. 411(6836):494–498.
18. Kulkarni JA, Cullis PR, van der Meel R. 2018. Lipid nanoparticles enabling gene therapies. Nucleic Acid Ther. 28(3):146–157.
19. Ambros V. 2004. The functions of animal microRNAs. Nature. 431(7006):350–355.
20. Calin GA, Hubé F, Ladomery MR, Delihas N, Ferracin M, Poliseno L, *et al*. 2024. The 2024 Nobel Prize in Physiology or Medicine: microRNA takes center stage. Non-Coding RNA. 10(6):62.
21. Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, *et al*. 2006. Small dsRNAs induce transcriptional activation in human cells. Proc Nat Acad Sci. USA. 103(46):17337–17342.
22. Doudna JA, Charpentier E. 2014. The new frontier of genome engineering with CRISPR-Cas9. Science. 346(6213):1258096.
23. Ledford H. 2015. CRISPR, the disruptor. Nature. 522(7554):20–24.
24. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, *et al*. 2016. Therapeutic genome editing *in vivo*. Nat Biotechnol. 34(3):328–333.
25. Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR. 2004. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med. 351(27):2805–2816.
26. Song KM, Lee S, Ban C. 2012. Aptamers and their biological applications. Sensors. 12(1):612–631.
27. Schlake T, Thess A, Fotin-Mleczek M, Kallen KJ. 2012. Developing mRNA-vaccine technologies. RNA Biol. 9(11):1319–1330.
28. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, *et al*. 2020. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 383(27):2603–2615.
29. Miller T, Cudkowicz M, Shaw PJ, Andersen PM, Atassi N, Bucelli RC, *et al*. 2020. Phase 1–2 trial of tofersen for SOD1 ALS. N Engl J Med. 383(2):109–119.
30. Miller TM, Cudkowicz ME, Genge A, Shaw PJ, Sobue G, Bucelli RC, *et al*. 2022. Trial of tofersen for SOD1 ALS. N Engl J Med. 387(12):1099–1110.
31. Hoy SM. 2024. Exagamglogene autotemcel: First approval. Mol Diagn Ther. 28(2):133–139.
32. Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, *et al*. 2024. Exagamglogene autotemcel for transfusion-dependent β-thalassemia. N Engl J Med. 390(18):1663–1676.
33. Kang C. 2023. Avacincaptad pegol: First approval. Drugs. 83(15):1447–1453.
34. Britton A. 2024. Use of respiratory syncytial virus vaccines in adults. Morb Mortal Wkly Rep. 73:6–11.
35. Yan YL, Zhao ZG. 2023. Clinical application and evaluation of marketed nucleic acid drugs. Chin J Clin Pharmacol. 24:3677–3681.
36. Khattak A, Carlino M, Meniawy T, Ansstas G, Medina T, Taylor MH, *et al*. 2023. Abstract CT001: A personalized cancer vaccine, mRNA-4157, combined with pembrolizumab *versus* pembrolizumab in patients with resected high-risk melanoma: Efficacy and safety results from the randomized, open-label Phase 2 mRNA-4157-P201/Keynote-942 trial. Cancer Res. 83(8_Suppl):CT001.
37. Bettini E, Locci M. 2021. SARS-CoV-2 mRNA vaccines: Immunological mechanism and beyond. Vaccines. 9(2):147.
38. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. 2014. Non-viral vectors for gene-based therapy. Nat Rev Genet. 15(8):541–555.
39. Juliano RL. 2016. The delivery of therapeutic oligonucleotides. Nucleic Acids Res. 44(14):6518–6548.
40. Wang J, Tan M, Wang Y, Liu X, Lin A. 2023. Advances in modification and delivery of nucleic acid drugs. J Zhejiang Univ Med Sci. 52(4):417–428.
41. Kurreck J. 2003. Antisense technologies: improvement through novel chemical modifications. Eur J Biochem. 270(8):1628–1644.
42. Shen W, Liang XH, Sun H, Crooke ST. 2015. 2'-Fluoro-modified phosphorothioate oligonucleotide causes rapid degradation of P54nrb and PSF. Nucleic Acids Res. 43(9):4569–4578.
43. Liao T, Li X, Tong Q, Zou K, Zhang H, Tang L, *et al*. 2017. Ultrasensitive detection of microRNAs with morpholino-functionalized nanochannel biosensor. Anal Chem. 89(10):5511–5518.
44. Naldini L. 2015. Gene therapy returns to centre stage. Nature. 526(7573):351–360.
45. Kotterman MA, Chalberg TW, Schaffer DV. 2015. Viral vectors for gene therapy. Annu Rev Biomed Eng. 17:63–89.
46. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, *et al*. 2018. Patisiran for hereditary transthyretin amyloidosis. N Engl J Med. 379(1):11–21.
47. Farooqi AA, Desai NN, Qureshi MZ, Librelotto DRN, Gasparri ML, Bishayee A, *et al*. 2018. Exosome biogenesis and functions. Biotechnol Adv. 36(1):328–334.
48. Kim SM, Yang Y, Oh SJ, Hong Y, Seo M, Jang M. 2017. Cancer-derived exosomes as CRISPR/Cas9 delivery platform. J Control Release. 266:8–16.

49. Zhang Y, Liu Q, Zhang X, Huang H, Tang S, Chai Y, *et al*. 2022. Exosome-mediated nucleic acid delivery for cancer therapy. J Nanobiotechnol. 20(1):279.
50. Son S, Nam J, Kim J, Kim S, Kim WJ. 2014. i-motif-driven Au nanomachines in programmed siRNA delivery for gene-silencing and photothermal ablation. ACS Nano. 8(6):5574–5584.
51. Kim H, Kim WJ. 2014. Photothermally controlled gene delivery by reduced graphene oxide–polyethylenimine nanocomposite. Small. 10(1):117–126.
52. Zhi F, Dong H, Jia X, Guo W, Lu H, Yang Y, *et al*. 2013. Functionalized graphene oxide mediated adriamycin delivery and miR-21 gene silencing to overcome tumor multidrug resistance in vitro. PLoS One. 8(3):e60034.