Molecular Therapy
Nucleic Acids
Review

Nucleic acid therapeutics: Past, present, and future

Sajid Naeem,[1,2] Ju Zhang,[1,2] Yang Zhang,[3] and Yu Wang[1,4]

[1]College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China; [2]College of Physics and Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China; [3]School of Biomedical Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen 518055, Guangdong, China; [4]Key Laboratory of Quantitative Synthetic Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

Nucleic acid therapeutics have become increasingly recognized in recent years for their capability to target both coding and non-coding sequences. Several types of nucleic acid modalities, including siRNA, mRNA, aptamer, along with antisense oligo, have been approved by regulatory bodies for therapeutic use. The field of nucleic acid therapeutics has been brought to the forefront by the rapid development of vaccines against COVID-19, followed by a number of approvals for clinical use including much anticipated CRISPR-Cas9. However, obstacles such as the difficulty of achieving efficient and targeted delivery to diseased sites remain. This review provides an overview of nucleic acid therapeutics and highlights substantial advancements, including critical engineering, conjugation, and delivery strategies, that are paving the way for their growing role in modern medicine.

INTRODUCTION

Traditional therapeutics involve the use of small-molecule drugs that interact with the active site of specific proteins, modulating their function. These drugs have been widely successful due to their cost-effective production, simple storage and transport at ambient temperatures, and convenient oral administration. However, small molecules primarily target proteins, which constitute only a small fraction of the genome, representing approximately 1%–2% of total DNA.[1] This approach generally overlooks the vast majority of the genome, including non-coding regions that house key regulatory components like enhancers, silencers, and non-coding RNAs. Furthermore, critical regulators associated with various pathological states are not easily susceptible to traditional small-molecule drugs and other conventional therapeutic methods. Research has demonstrated that merely 20% of the translated proteins can be effectively targeted with small molecules.[2] As our understanding of the molecular mechanisms driving disease progression deepens, therapeutic strategies have the potential to be significantly broadened by focusing on genomic targets rather than just translated proteins. The genetic codes found in DNA and RNA are simpler and more linear compared with the three-dimensional structures of proteins, making them more accessible for targeted interventions using complementary nucleic acid (NA) molecules. Additionally, NA molecules, as therapeutic agents, are more easily screened, synthesized, and modified for precise adjustments, if necessary, thus demonstrating significant potential in modern therapeutics.[3]

Initially viewed as unstable intermediate products within the central dogma of molecular biology, RNA molecules have since been recognized for their diverse and significant roles through subsequent investigations.[4,5] The multifaceted functions of RNA in cellular physiology have become increasingly evident. The advent of NA-based drugs has introduced numerous advantages over traditional pharmaceuticals, such as expedited and cost-effective screening and synthesis processes that can target both coding and non-coding sequences, thereby substantially expanding the range of targets. These drugs offer flexibility in design and customization for personalized therapeutics, enabling rapid screening and development of sequences against emerging pathogens. In the past 5 years, NA therapeutics have experienced unprecedented progress, becoming a major focus of scientific research. During this time, 13 of the 21 currently approved NA-based therapies were approved, underscoring the rapid progress and transformative potential of this area. Notably, this period has seen the development of the fastest vaccines against coronavirus disease 2019 (COVID-19), which were pivotal in managing the global pandemic. Additionally, the first-ever approval of a CRISPR-Cas9-based therapy, Casgevy, has marked a significant milestone in gene editing technology. The recent approval of an aptamer Izervay (avacincaptad pegol) further underscores the versatility and expanding horizons of NA therapeutics. These advancements collectively highlight the rapid evolution and transformative impact of NA-based treatments in modern medicine.

Antisense oligonucleotides

Antisense oligonucleotides (ASOs) are short, engineered, single-stranded DNA, RNA, or a combination of both molecules that can bind to specific target RNA sequences through Watson-Crick base pairing and represent the simplest yet most effective strategy to manipulate gene expression. ASOs bind to complementary RNA targets via Watson-Crick hydrogen bonds to inhibit translation.[6] ASOs can modulate gene expression or modify pre-mRNA splicing via

https://doi.org/10.1016/j.omtn.2024.102440.

Correspondence: Yang Zhang, School of Biomedical Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen 518055, Guangdong, China.
E-mail: zhangyang07@hit.edu.cn
Correspondence: Yu Wang, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China.
E-mail: yuwang@post.harvard.edu

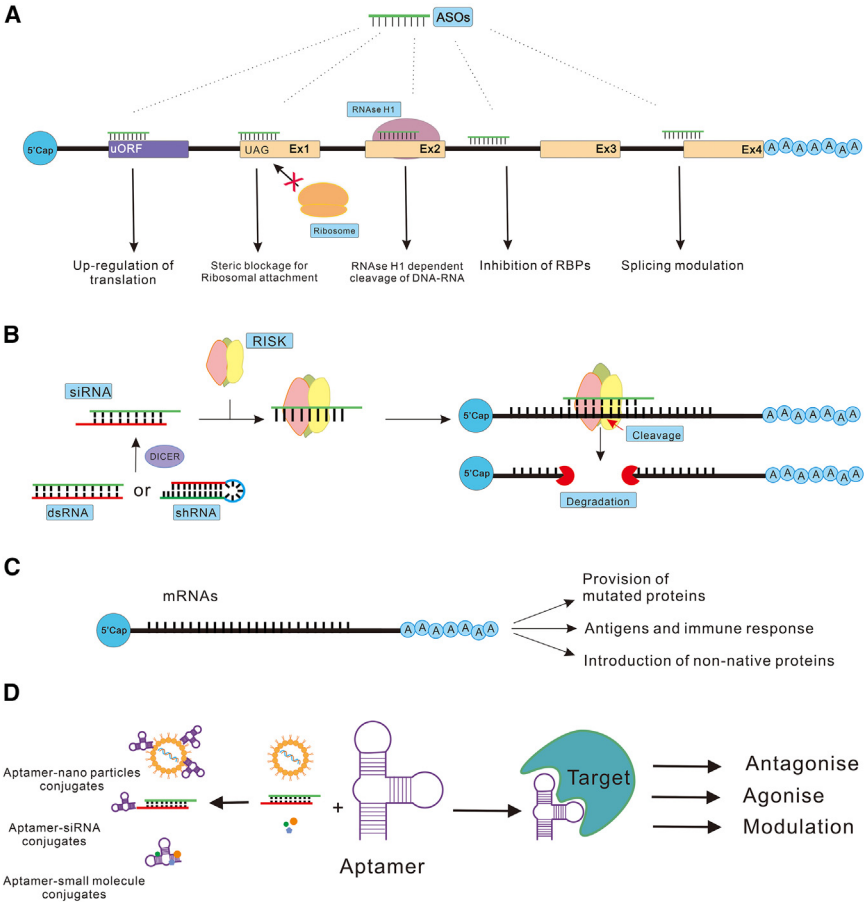

Figure 1. Overview of nucleic acid-based therapeutics

(A) ASOs target sites and subsequent modulation outcomes. (B) siRNA-mediated RNA interference pathway detailing Dicer processing of dsRNA to siRNA, leading to mRNA cleavage and degradation via the RISC complex. (C) Structure and potential outcomes of exogenous mRNAs. (D) Aptamer-based conjugates targeting specific molecules (left) and potential molecular interactions of aptamers at their specific targets (right).

distinct mechanisms depending on their chemistry, binding sequences, and target sites. For instance, ASOs can be designed to complement the target RNA, forming an ASO-RNA hybrid recognized by endogenous RNase H1, which subsequently cleaves the target mRNA.[7] RNase H1 is distributed in both the nucleus and the cytoplasm, extending the targeting of immature pre-mRNAs in the nucleus and long non-coding RNAs in the cytoplasm.[8,9] Alternatively, oligos can be designed to bind around the AUG start site or untranslated sequences, sterically blocking the binding of RNA-binding protein complexes, such as ribosomal subunits and RNA-binding proteins, thereby suppressing the translation of target mRNA.[10] ASOs are categorized as either RNase H1-dependent or RNase H1-independent depending on their mechanism of action.[11] Interestingly, ASOs can also enhance protein translation by targeting the upstream open reading frames (uORFs) that inhibit the expression of the main ORFs (Figure 1A).[12]

The concept of using ASOs for gene expression modulation was first proposed by Stephenson and Zamecnik in 1978, who demonstrated the inhibition of Rous sarcoma viral replication *in vitro* using synthetic tri-decamer ASOs.[13] Interestingly, the mechanism of RNase H1 enzyme-mediated cleavage was discovered the following year.[14] The initial development of ASOs was hindered by several technical challenges at the time, such as the synthesis of desired sequences, the lack of efficient delivery systems, and the rapid degradation by native nuclease systems in cells.[15] In order to protect from nucleases and to improve stability, significant chemical modifications have been made to ASOs, resulting in their success in therapeutics. Key milestones contributing to the success of ASOs include several important modifications. The concept of the phosphorothioate (PS) backbone dates back to 1966,[16] followed by the introduction of 2′-O-Methoxyethyl (MOE),[17] peptide nucleic acids (PNAs), and phosphorodiamidate morpholino oligomers (PMOs) in the 1990s,[18,19] whereas the early 2000s saw the development of locked nucleic acids (LNA).[20] These modifications significantly improved the pharmacokinetic properties, target binding affinity, and resistance to enzymatic degradation, enhancing the therapeutic potential and safety profile of ASOs. Moreover, advancements have been made for efficient and tissue-specific delivery strategies by employing conjugation approaches, such as coupling ASOs with cell-penetrating peptides (CPPs),[21] antibodies,[22] or aptamers.[23] However, ASOs face several critical challenges, including the rapid response of RNases leading to the degradation of exogenous RNAs. Furthermore, negatively charged RNA molecules have difficulty crossing the hydrophobic cytoplasmic membrane. Additionally, the strong immunogenicity of exogenous RNAs can cause cell toxicity, impairing their effectiveness as therapeutic agents. Limited tissue penetrance further complicates specific tissue targeting, posing an additional challenge to the effective delivery of ASOs to target sites.

Small interfering RNA molecules

Double-stranded small interfering RNA molecules (siRNAs) share a similar mechanism of action as ASOs by specifically hybridizing to the targeted sense strand RNA via Watson-Crick hydrogen base pairing.[24] These siRNAs, also known as silencing RNAs, are effective at interfering with gene expression by causing the degradation or inhibition of target RNA molecules, making them a valuable tool in molecular biology and medical research for gene knockdown experiments.

The siRNA duplexes originate from naturally transcribed or artificially introduced precursor siRNAs, which range from 30 to more than 100 base pairs (bp) in length. The endogenous Dicer enzyme processes these precursors into 20- to 30-bp-long siRNAs with two-base 3′ overhangs, which interact with the endogenous RNA-induced silencing complex (RISC) to elicit RNA interference (RNAi). The endonuclease Argonaute 2 (AGO2) component of the RISC cleaves the sense strand of the duplex, leaving the antisense strand intact, then guides the active RISC to its target mRNA. AGO2 subsequently cleaves the phosphodiester backbone of the target mRNA. The antisense strand is usually fully complementary to the coding region of the target mRNA, allowing siRNA to knock down one specific target gene.[25,26] Being double-stranded, siRNAs remain inactive until the transactivation-responsive RNA-binding protein (TRBP) activates them by loading into AGO2, which subsequently separates the passenger (sense) strand and allows the binding of the guide (antisense) strand to catalytic AGO2 (Figure 1B).[27]

In 1998, 2 decades after ASOs were first discovered, Fire et al. made a major breakthrough in molecular biology by discovering that double-stranded RNAs, known as siRNAs, were highly effective at inhibiting target genes. Their experiments showed that siRNAs could efficiently block the expression of genes such as unc-22 and unc-54, which play roles in muscle movement, fem-1, a key regulator in sex determination, and the basic-helix-loop-helix (bHLH) transcription factor hlh-1 in the nematode *C. elegans*. The remarkable aspect of this discovery was that the inhibitory effect of siRNAs could be passed on to subsequent generations.[28] Moreover, the siRNAs showed more resistance to the native nucleases of the cell, and only a few copies of the siRNAs were needed to result in significant inhibition of the target. Three years later, Elbashir et al. demonstrated the silencing of endogenous and exogenous genes in mammalian cell systems using chemically synthesized 21-bp double-stranded siRNAs and predicted the therapeutic potential of 21-bp synthetic RNA duplexes.[27] Again, just like ASOs, the success of siRNA in therapeutics remained dependent on improved delivery mechanisms and enhanced stability measures.[24] Due to their double molecular weight and negative charge compared with ASOs, siRNAs face more difficulty in crossing the cell membrane to reach target sites. However, this difficulty can be mitigated by a number of strategies, including siRNA-encapsulating lipid nanoparticles (LNPs), polymer-based delivery systems such as polyethylenimine (PEI) and polyethylene glycol (PEG), the utilization of CPPs such as TAT peptides, and nanoparticle-based delivery systems.[29] Additionally, antibody-mediated delivery, vector-based siRNA delivery,[30] and organ-specific and tissue-specific delivery approaches are utilized for efficient targeting.[31] For instance, GalNAc-siRNA conjugates represent a significant milestone in achieving targeted delivery of siRNA to the liver,[32] which have been utilized in the development of all Food and Drug Administration (FDA)-approved siRNA therapeutics except patisiran (Table 1). The stability concerns of siRNA due to inherent chemical properties were overcome by introducing chemical modifications capable of making them stable in the cellular environment, thus advancing siRNA-based drugs.

The continuous progress and efforts in developing RNA-based drugs finally resulted in the approval of the first siRNA-based drug in 2018, named patisiran (Onpattro, Alnylam Pharmaceuticals). It is targeted to degrade transthyretin (TTR) mRNA, which mediates polyneuropathy in adult patients.[42] The second siRNA-based drug, named givosiran (Givlaari, Alnylam Pharmaceuticals), was approved in 2019 and is being used to treat acute hepatic porphyria. The drug targets aminolevulinate synthase 1 mRNA in the liver, reducing the levels of disease-causing neurotoxic intermediates aminolevulinic acid and porphobilinogen.[52,43] Many more siRNA drugs are in the pipeline and in trial phases. For instance, recently, an siRNA against the hepatitis B virus (HBV), encapsulated in an ionizable lipidoid nanoparticle (RBP131), has been developed and tested, producing promising results by reducing the viral DNA, RNA, and antigens.[53]

mRNAs

mRNAs are known for their intermediary role in the central dogma of molecular biology, carrying transient blueprints of the genome. However, the therapeutic potential of mRNAs was brought to light in the last decade of the 20th century by introducing exogenous mRNAs into animals to express desired proteins. In 1990, Wolf et al. injected mouse skeletal cells with synthesized mRNA sequences encoding luciferase, chloramphenicol acetyltransferase, and beta-galactosidase, successfully inducing the expression of these proteins.[54] This breakthrough opened a new horizon for RNA therapeutics, where synthetic or purified mRNAs could be introduced to compensate for mutated or nonfunctional proteins in animals. One such example is the injection of synthetic or purified vasopressin mRNA from wild-type rats into the hypothalamus of vasopressin-deficient Brattleboro rats, resulting in the transient reversal of diabetes insipidus.[55]

In mRNA therapeutics, the sequences can be easily modified, allowing the personalization of NA therapy. These therapies can be divided into the following subgroups: (1) replacement therapy, where mRNAs are administered to compensate for a defective gene or protein, or to supply therapeutic proteins; (2) vaccination, where mRNAs encoding specific antigens are administered to trigger an immune response; (3) cell therapy, where mRNA is transfected into cells *ex vivo* to alter cell phenotype or function, and these cells are then delivered into the patient (Figure 1C).[11] The development of mRNAs as therapeutics has faced several challenges, including mRNA stability, immunogenicity, delivery, and scalability. However, significant progress has been made to overcome these challenges. Unmodified mRNA molecules are highly susceptible to degradation by cellular ribonucleases, limiting their therapeutic potential, as they need to remain intact for a sufficient duration to be translated into functional proteins. To improve the stability of mRNAs against native ribonucleases, nucleosides are modified with naturally occurring chemical groups, such as pseudouridine,[56,57] 5-methylcytidine,[58,59] and 1-methylpseudouridine,[60] which are incorporated into mRNA molecules. These modifications enhance mRNA stability by reducing its recognition and degradation by cellular ribonucleases.[61,62] Additionally, structural modifications at the 3′ and 5′ ends, as well as in the untranslated regions (UTRs), help improve stability and reduce immunogenic responses.[63] The

Table 1. List of approved NA therapeutics (up to August 2024)

RNA type	Drug name(s)	Target profile	Chemical modification and carrier	Developers	Administration	Year approved	Reference
ASO	Fomivirsen (Vitravene, ISIS 2922)	Eye, cytomegalovirus (CMV) retinitis: Bind and degrade UL123 mRNA and inhibit CMV protein IE2	2′-H	Ionis	i.v.t	1998, FDA, EMA Withdrawn 2002	Geary et al.[33]
	Mipomersen (Kynamro™, ISIS 301012)	Liver, homozygous familial hypercholesterolemia, bind and degrade ApoB-100 mRNA	Gapmers, 2′-MOE	Kastle Therapeutics and Ionis	s.c.	2013	Santos et al.[34]
	Nusinersen (Spinraza, ISIS 396443)	CNS, spinal muscular atrophy: splice the pre-mRNA of SMN2	2′-MOE	Ionis	i.t	2016	Mercuri et al.[35]
	Eteplirsen (Exondys 51, AVI-4658)	Muscles, Duchenne muscular dystrophy: Splice exon 52 of DMD	2′-MOE, PMO	Sarepta	i.v	2016	Mendell et al.[36]
	Inotersen (Tegsedi, ISIS 420915)	Liver, familial amyloid polyneuropathy: Inhibit translation of TTR mRNA translation	2′-MOE,	Ionis	s.c.	2018	Benson et al.[37]
	Milasen	CNS, Mila Makovec's CLN7 gene associated with Batten disease: Splice the CLN7 mRNA	–	Boston Children's Hospital	i.t	2018, Approved for personalized use	Kim et al.[3]
	Golodirsen (Vyondys 53™, SRP-4053)	Muscles, Duchenne muscular dystrophy: Splice exon 53 of DMD	2′-MOE, PMO	Sarepta	i.v	2019	Scaglioni et al.[38]
	Viltolarsen (Viltepso, NS-065, NCNP-01)	Muscles, Duchenne muscular dystrophy: Splice exon 53 of DMD	2′-MOE, PMO	NS Pharma	i.v	2020	Clemens et al.[39]
	Casimersen (SRP-4045, Amondys 45™)	Muscles, Duchenne muscular dystrophy: Splice exon 45 of DMD	PMO	Sarepta	i.v	2021	Shirley et al.[40]
	Eplontersen (Wainua)	Muscles, polyneuropathy of hereditary transthyretin-mediated amyloidosis	GalNAc-conjugated	AstraZeneca and Ionis Pharmaceuticals	s.c	2023	Coelho et al.[41]

(*Continued on next page*)

Table 1. *Continued*

RNA type	Drug name(s)	Target profile	Chemical modification and carrier	Developers	Administration	Year approved	Reference
siRNA	Patisiran (Onpattro, ALN-TTR02, ONPATTRO™)	Liver, polyneuropathy: Inhibit translation of TTR mRNA translation	PS, 2′-O-Me, 2′- F, LNP	Alnylam	i.v.	2018	Kristen et al.[42]
	Givosiran (Givlaari, ALN-AS1)	Liver, acute hepatic porphyria: Targeting ALAS1 mRNA and downregulation	PS, 2′-O-Me, 2′- F, GalNAc	Alnylam	s.c.	2019	Balwani et al.[43]
	Lumasiran (OXLUMO, ALNGO1)	Liver, primary hyperoxaluria type 1: Targeting HAO1 mRNA and downregulation of glycolate oxidase	PS, 2′-O-Me, 2′- F, GalNAc	Alnylam	s.c.	2020	Liebow et al.[44]
	Inclisiran (LEQVIO, ALN-PCSSC)	Liver, atherosclerotic cardiovascular disease: Targeting PCSK9 mRNA and downregulation of proprotein convertase subtilsin/kexin type 9	PS, 2′-O-Me, 2′- F, GalNAc	Novartis	s.c.	2021	Lamb et al.[45]
	Vutrisiran (ALN-TTRSC02)	Liver, hATTR treatment, TTR mRNA	PS, 2′-O-Me, 2′- F, GalNAc	Alnylam	s.c.	2022	–
	Nedosiran (RIVFLOZA)	Primary hyperoxaluria	PS, 2′-O-Me, 2′- F, GalNAc	Dicerna	s.c.	2023	Goldfarb et al.[46]
Aptamers	Pegaptanib (Macugen)	Eye, macular degeneration: Binding with hairpin loop of VEGF165 and subsequent inactivation	Pegylated, all PO, 2′-F, and 2′-OMe; G and A methylated	Bausch + Lomb	i.v.t	2004	Gragoudas et al.[47]
	Izervay (avacincaptad pegol)	Eye, complement C5 inhibitor to treat geographic atrophy (GA)	PEGylated oligonucleotide	Iveric Bio + Astellas Pharma	i.v.t	2023	Jaffe et al.[48]
mRNA	BNT162b2	Immune system, COVID-19 vaccine: SARS-CoV-2 S antigens' expression	Nucleosides-modified, Lipid nanoparticle-formulated	BioNTech and Pfizer	IM	2020	Polack et al.[49]
	mRNA-1273	Immune system, COVID-19 vaccine: SARS-CoV-2 S antigens' expression	Lipid nanoparticle-formulated	Moderna	IM	2020	Baden et al.[50]
CRISPR-Cas9	Casgevy (exagamglogene autotemcel)	Blood, editing of CD34+ HSPCs using CRISPR-Cas9	–	Vertex Pharmaceuticals Inc + CRISPR Therapeutics	i.v.	2023	Frangoul et al.[51]

i.v.t, intravitreal; i.m, intramuscular; i.t, intrathecal; i.v., intravenous; s.c., subcutaneous.

large, negatively charged size of mRNA molecules poses challenges for crossing cell membranes and reaching the translation sites in the cytoplasm. Similar to the aforementioned smaller NA molecules, LNPs,[64] viral vectors,[65] and classical electroporation techniques can be employed to overcome this challenge.

Circular RNA molecules

Circular RNAs (circRNAs) are an emerging class of RNA molecules with unique properties and therapeutic potential. Unlike linear RNAs, circRNAs form covalently closed continuous loops without 5′ caps or 3′ poly(A) tails, making them resistant to exonuclease-mediated degradation and remarkably stable in cellular environments.[66] circRNAs are produced from precursor mRNA (pre-mRNA) through back-splicing, a process in which a downstream 5′ splice site is joined to an upstream 3′ splice site.[67] These molecules are highly abundant, evolutionarily conserved, and exhibit tissue- and developmental stage-specific expression patterns, suggesting their critical regulatory roles in various biological processes.[68]

One of the key mechanisms by which circRNAs exert their functions is through sponging microRNAs (miRNAs).[69,70] By harboring multiple miRNA response elements (MREs), circRNAs can act as competing endogenous RNAs (ceRNAs), sequestering miRNAs and thereby regulating the expression of miRNA target genes.[70] For instance, circHIPK3 has been shown to sponge miR-124, thereby influencing cell proliferation and apoptosis, which is highly relevant in cancer biology.[71] In addition to miRNA sponging, circRNAs interact with RNA-binding proteins (RBPs) to modulate their activity and localization.[72] Some circRNAs can also be translated into functional peptides, expanding their functional repertoire.[73] Stability and low immunogenicity of circRNAs make them attractive candidates for therapeutic applications.[74] Unlike linear RNAs, circRNAs do not trigger innate immune responses via pattern recognition receptors such as Toll-like receptors (TLRs), which often limit the use of linear RNAs in clinical settings.[75] However, efficient delivery of circRNA therapeutics remains a challenge.

circRNA-based drugs are currently in the early stages of development, with several preclinical studies demonstrating their potential.[76] For example, a synthetic circRNA designed to sponge miR-21, an oncogenic miRNA implicated in multiple cancers, effectively suppressed tumor growth in mouse models.[77,78] Similarly, circRNAs encoding functional peptides have been explored in the context of cardiovascular diseases. A recent study utilized synthetic circRNAs to produce cardioprotective peptides,[79] demonstrating therapeutic benefits in ischemia-reperfusion injury models. circRNA-based therapies have been investigated in neurodegenerative diseases, viral infections, and immune disorders.[80,81] For instance, circRNAs targeting miR-122, a liver-specific miRNA crucial for hepatitis C virus (HCV) replication,[82] have shown promise in reducing liver fibrosis.[83] Moreover, engineered circRNAs have been explored as vaccines, with studies showing their ability to elicit robust antigen-specific immune responses against pathogens.[84]

Micro RNA molecules

miRNAs are small, non-coding RNA molecules of approximately 20–24 nucleotides that regulate gene expression at the post-transcriptional level. They function by binding to complementary sequences in the 3′ untranslated regions (UTRs) of target messenger RNAs (mRNAs), leading to either translational repression or mRNA degradation through the RISC. The Argonaute protein (AGO) is a central component of RISC, guiding the miRNA to its target mRNA for gene silencing.[85] Unlike siRNAs, miRNAs typically exhibit partial complementarity to their target, allowing them to regulate multiple genes simultaneously. This versatile mechanism underpins their critical roles in various physiological processes, including development, differentiation, apoptosis, and immune responses.[86]

The therapeutic potential of miRNAs was first recognized in the context of cancer, where miRNAs can act as either tumor suppressors or oncogenes (oncomiRs). For instance, the tumor-suppressive miR-34 family targets genes involved in cell cycle regulation and apoptosis,[87] while oncomiRs like miR-21 promote tumor progression by downregulating tumor suppressors.[88] The development of miRNA-based therapeutics involves either miRNA mimics to restore the function of downregulated miRNAs or antagomiRs to inhibit overexpressed miRNAs. One prominent example is MRX34, a liposomal formulation of miR-34a mimic, which entered clinical trials as a treatment for various cancers but faced challenges related to immune activation.[89] RG-125 (AZD4076), a GalNAc-conjugated antagomiR targeting miR-103/107, is being developed for metabolic diseases.[90] These developments underscore the transformative potential of miRNAs across diverse therapeutic areas.

Aptamers

Aptamers are short single-stranded DNA or RNA molecules with specific tertiary structures that enable them to bind to a variety of targets, such as proteins, peptides, carbohydrates, organelles, and even cells. The first aptamers were reported in 1990 by two independent groups through a technique known as Systematic Evolution of Ligands by EXponential enrichment (SELEX).[91,92] Aptamers are more versatile compared with NA oligonucleotides, which primarily serve sequence-specific inhibitory roles. In contrast, aptamers can bind to a wide variety of targets with high specificity and affinity. This binding capability arises from their ability to adopt well-defined three-dimensional structures, such as hairpins, loops, or pseudoknots, that are crucial for their interaction with target molecules. These structural features allow aptamers to achieve exceptional selectivity, even distinguishing between closely related molecular targets. These properties make aptamers valuable tools for modulating protein functions, facilitating targeted drug delivery systems, and serving as molecular recognition elements in diagnostics and therapeutic applications (Figure 1D).[93]

The therapeutic potential of aptamers was recognized because of their ability to act as antagonists, leading to the identification of numerous antagonist aptamers. For instance, a tumor-targeting RNA aptamer has been found to inhibit p68, an RNA helicase that is upregulated

in colorectal cancer.[94] Moreover, the first FDA-approved aptamer drug, pegaptanib, specifically binds to vascular endothelial growth factor-A (VEGF-A), inhibiting its interaction with receptors and preventing angiogenesis.[47] More recently, on August 4, 2023, the FDA approved Izervay (avacincaptad pegol) for the treatment of geographic atrophy (GA) caused by advanced dry age-related macular degeneration (AMD). Izervay is a complement C5 inhibitor that is injected directly into the eye in a doctor's office, once a month or every other month. It works by reducing the immune response to help prevent damage to retinal cells. In clinical trials, Izervay significantly reduced the mean rate of GA progression by over 27% in the first year.[48] Izervay is the only approved GA treatment to show a statistically significant reduction in progression after 12 months, although it has not been shown to improve eyesight or restore lost vision.

Aptamers can also play a modulatory role in target protein function. For example, IR-A62, an insulin receptor (IR) binding aptamer, acts as a biased agonist that preferentially induces Y1150 monophosphorylation of IR in the absence of insulin. At lower concentrations, it promotes insulin binding by acting as a positive allosteric modulator (PAM) agonist, while at higher concentrations, it acts as a negative allosteric modulator (NAM) agonist and competes with insulin for IR binding.[95] Since aptamers are highly specific for their targets, they can be utilized to improve the efficiency of targeted drug delivery systems. For instance, an endoglin aptamer (YQ26)-modified fluorescent silica nanoparticle (YQ26-FsiNPs) has been reported for high efficiency *in vivo* tumor imaging, monitoring, and drug delivery.[96]

Another unique way in which aptamers can modulate gene expression is by acting as multidirectional riboswitches. The binding of aptamers with ligands induces conformational changes in their tertiary structure, a property that can be exploited to design ligand-induced aptamer switches for multidirectional expression systems.[97,98] A number of natural and synthetic aptamer switches have been identified to modulate almost every process of mRNA turnover. For instance, Vogel et al. employed one of the most commonly used tetracycline-binding riboswitches for exon skipping.[99] In another study, the expression of GFP was controlled at the translational level by inserting and characterizing tetracycline-binding riboswitches at the 5′-UTR of a GFP-encoding mRNA.[100] Riboswitches have also shown their potential to regulate AAV-mediated transgene expression *in vivo*. The tetracycline-dependent ribozyme K19 has shown promising multiorgan functionality, robust regulation, reversibility, and the capacity for fine-tuning and repeated induction of gene expression.[101]

Despite all this intrinsic value, there are a few drawbacks associated with aptamers. Like linear RNA oligonucleotides, unmodified aptamers are quickly degraded by native nucleases in the cell system, often within seconds to an hour.[102,103] Due to their small size (<5 nm) and light weight (6–30 kDa),[104] aptamers are prone to leak through renal filtration.[105] These factors hinder the therapeutic potential of aptamers. To pave the way for the clinical application of aptamer-based therapeutics, extensive modifications of aptamer chains and conjugations with additional moieties, such as PEG, to extend their half-life, are therefore necessary.

The issue of aptamer elimination by renal filtration can be mitigated by combining aptamers with heavier compounds, such as PEG,[106] liposomes,[107] peptides,[108] cholesterols,[109] and a number of nano-materials.[110,111] Aptamers offer great potential in therapeutics, particularly when used in complement with monoclonal antibodies, because they can be produced via cost-effective chemical synthesis, are easier to modify, and elicit less immunogenicity compared with antibodies.[112]

Ribozymes

During the early 1980s, researchers unearthed yet another role of RNA molecules: catalysis. Guerrier-Takeda et al. revealed that the catalytic capability of purified ribonucleases from prokaryotes was entirely dependent on the RNA moieties but not the protein parts.[113] Another study provided evidence for the catalytic activity in self-splicing ribosomal RNA, demonstrating that a shortened form of the intervening sequence from *Tetrahymena thermophila* could act as an enzyme *in vitro* in a sequence-specific manner.[114] These discoveries encouraged more researchers to explore the catalytic capabilities of other RNA molecules. Consequently, hammerhead ribozymes were discovered in 1987 and hairpin ribozymes in 1990, with the former being well studied and used in therapeutics.[115,116] The hammerhead ribozyme is mainly divided into two parts: (1) three hybridizing helices, which flank and bind the antisense strand of target RNA, and (2) a catalytic core that mediates the cleavage reaction.[117] The catalytic core cleaves the RNA at a specific site generally described as XUN, where X is any base. Once the cleavage occurs, the two cleaved RNAs are unstable and prone to degradation by native nucleases, and the hammerhead ribozymes detach and are ready to target another copy of such sequences. Thus, hammerhead ribozymes are naturally suited for suppressing target sequences.

At the time of their discovery, HIV was one of the biggest medical challenges. A study employed synthetic hammerhead ribozymes to target the HIV sequence and demonstrated a substantial decrease in HIV gag RNA sequences in human cell lines.[118] This was the first study indicating the therapeutic potential of ribozymes. Around the same time, another study successfully targeted and cleaved the c-fos oncogene mRNA with ribozymes, confirming the working model of the newly presented idea of gene suppression.[119] Ribozymes offer an advantage that they perform their function independent of any other cellular protein.[120] There were some clinical trials regarding the use of ribozyme therapeutics, but most were abandoned due to insufficient efficiency. One example is RPI.4610 (Angiozyme), an anti-VEGFR-1 ribozyme used in combination with carboplatin and paclitaxel to treat solid tumors with encouraging results.[121] However, in other cancers, such as metastatic breast cancer, its efficacy was not up to the mark, and it was discontinued from further development.[122]

Several challenges hinder the clinical application of ribozymes. First, ribozymes often exhibit limited stability in biological environments, where they are prone to degradation by nucleases. This necessitates the development of chemically modified ribozymes or effective delivery systems to enhance their stability. Early studies showed that chemical modifications of hammerhead ribozymes, such as 2′ modified pyrimidine nucleosides and phosphonothioates, significantly increased ribozyme stability.[123] Recently, experiments with structural modifications have also increased the stability and target specificity of ribozymes. For instance, the addition of a hairpin motif and the formation of trans-Hoogsteen interactions to mimic exogenous ribozymes with highly active native ribozymes resulted in an increased reduction of influenza A virus mRNAs.[124] Additionally, achieving efficient cellular uptake and targeted delivery remains a critical obstacle. The complexity of intracellular environments can impede ribozyme functionality, necessitating sophisticated delivery vectors such as lipid nanoparticles or viral vectors. Furthermore, the specificity of ribozyme activity requires precise sequence recognition, which can be complicated by the presence of similar or mutant RNA sequences in the target cells. Overcoming these challenges requires ongoing advancements in RNA chemistry, delivery technologies, and a deeper understanding of ribozyme interactions within cellular contexts, paving the way for their successful therapeutic application.

RNA editing and modulation

RNA editing and modulation have emerged as exciting fields in NA therapeutics, offering precise and targeted manipulation of RNA molecules for the correction of genetic mutations and modulation of gene expression. Adenosine deaminases acting on RNA (ADARs) have long been known and are the most abundant base editors in humans, with adenosine-to-inosine (A-to-I) conversion accounting for more than 90% of all editing events.[125] Other less prevalent RNA editing events include cytosine-to-uracil (C-to-U) editing, which involves the deamination of cytosine to uracil and plays a role in modifying transfer RNA (tRNA) and mitochondrial RNA sequences. The enzyme responsible for catalyzing C-to-U RNA editing is called apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC), and activation-induced cytidine deaminase (AID). These enzymes belong to the APOBEC/AID cytidine deaminase family.[126] The reversal of C-to-U, i.e., U-to-C, is mediated by uracil terminal uridylyl transferase, also known as TUTase or TUTase-like enzyme. TUTases are a group of enzymes that catalyze the addition of cytosine residues opposite uracil (U) residues in RNA molecules.[127]

ADARs have the capability of converting A to I in coding regions as well as in Alu repeats containing non-coding regions of double-stranded RNAs, thereby modulating gene expression through various regulatory mechanisms.[128] Inosine, functionally similar to guanosine (G), leads to altered RNA structures and subsequent changes in protein expression.[129] In the context of RNA therapeutics, ADAR-based editing offers a unique approach to correct disease-causing mutations or modulate gene expression. By targeting specific RNA transcripts, ADARs can be used to restore proper protein function or fine-tune gene expression levels, providing a versatile tool for treating various genetic disorders. Furthermore, recent advancements in genome editing technologies, such as CRISPR-Cas systems, offer enhanced precision and efficiency in ADAR-mediated RNA editing, paving the way for potential clinical applications.[130] A simpler strategy for targeted RNA editing can be achieved through ectopically expressed R/G motif-bearing synthetic guide RNAs, which are coupled with ADARs.[131,132] Moreover, a number of RNA-targeting modalities including λN-peptide[133] and an SNAP-tag[134] and even Cas13 protein[130] have been fused with ADARs for targeted RNA editing. However, these strategies face challenges such as undesired off-target effects,[135] immunogenicity, and delivery.[136,137] To avoid the challenges associated with employing exogenous ADARs for RNA editing, chemically optimized ASOs have been synthesized to recruit endogenous ADARs, a method referred to as recruiting endogenous ADARs to specific transcripts for oligonucleotide-mediated RNA editing (RESTORE).[138] A similar approach employed short engineered ADAR-recruiting RNAs (arRNAs) to recruit native ADARs for specific RNA editing, named leveraging endogenous ADARs for programmable editing of RNA (LEAPER).[139] Both approaches significantly improved editing efficiency with minimal global off-target effects. More recently, the same group reported LEAPER 2.0, an enhancement of LEAPER, by employing covalently closed circular arRNAs (cir-arRNAs), which demonstrated around a 3-fold increase in editing efficiency *in vitro* and *in vivo*.[140]

The mechanism of A-to-I RNA editing is mediated by hydrolytic deamination of the adenine base. ADARs specifically recognize double-stranded RNA from various RNA moieties, including pre-mRNA, pri-mRNA, pri-siRNA, lncRNA, and transposons. Since these non-coding elements have a strong impact on the expression of translated proteins, ADARs hold potential as therapeutic tools. ADARs naturally and nonspecifically bind to double-stranded RNAs and even tertiary RNA structures, modifying imperfectly paired double-stranded RNAs.[141–143] However, more recently, a number of strategies have been employed for site-specific and precise RNA editing. These strategies include programmable site-directed deployment of ADARs using chemically manipulated oligonucleotides and gRNAs.[138,144–146] With the breakthrough discovery of type VI CRISPR-Cas systems and more efficient Cas13 variants, which exclusively target single-stranded RNAs,[147,148] a new avenue for more precise and efficient RNA manipulation has been opened.[149]

DNA targeting provides permanent changes to the genome and comes with concerns about off-target effects and irreversibility. In this regard, RNA editing offers advantages over DNA editing in therapeutics.

Challenges in NA therapeutics

NA therapeutics hold immense promise for treating a wide range of diseases, as evidenced by the approval of several therapeutic agents; however, these therapeutics face significant challenges that must be overcome to achieve successful clinical translation. One of the critical challenges in the field of NA therapeutics is the efficient delivery of

NA molecules to target cells and tissues. The fragile nature of RNA, its vulnerability to nucleases, and the presence of multiple biological barriers hinder the effectiveness of delivery strategies. Unintended interactions between NA therapeutics and non-targeted cells, as well as the unintended activation of immune responses, can lead to adverse effects and limit therapeutic efficacy. Moreover, the regulatory landscape for NA therapeutics is continually evolving. The regulatory approval processes for NA-based drugs require careful consideration, with guidelines and standards adapting to advancements in the field. To address these challenges, ongoing progress in several key areas is essential to ensure the safe and effective utilization of NA therapeutics, which is discussed in detail in this section.

Screening of aptamers

To advance the success of NA therapeutics, it is imperative to establish efficient and accurate screening methods that can reliably assess NA molecules for their intended purposes. The design and synthesis of linear and simpler NA sequences, such as ASOs, siRNAs, and mRNAs, targeting specific DNA or RNA molecules, have become more accessible with the advent of sophisticated software and online databases. However, aptamers exhibit complex behavior due to their three-dimensional structural variations and folding, which can substantially alter their affinity for targets. These molecules are initially screened from large *in silico* pools and subjected to rigorous screening phases based on their specificity to particular targets. Despite this, the screened molecules may not function properly in their natural environment for several reasons. Therefore, intensive screening and contingent selection of efficient molecules must be performed through actual experiments under native conditions. Different strategies are utilized to screen various types of NA molecules, which will be discussed in this section.

SELEX

Systematic Evolution of Ligands by EXponential enrichment (SELEX) has been used to screen aptamers since the 1990s.[91,92,150] Briefly, SELEX is designed to screen aptamers from very large random sequence libraries, which may comprise over 1 trillion different sequences. These sequence libraries are challenged to bind to target sequences under set physicochemical conditions. The molecules that bind to the target are isolated and subjected to further, more stringent selection criteria.[151] This cycle may be repeated several times to isolate the most efficient molecules for desired targets. The aptamers selected through this process are relatively easier to separate due to their distinct three-dimensional folding and their *in vitro* replication preference.[150,152] Various SELEX variants have been developed and optimized for the selection of a wide range of nucleic acids, and these are summarized elsewhere.[152]

Although SELEX has long been used for screening single-stranded DNA (ssDNA) or RNA aptamers,[153] there are some shortcomings associated with this technique. For instance, purified-protein-based SELEX may not accurately mimic the native conformations of target proteins in their natural cellular environment.[154] On the other hand, cell-based SELEX screening can overcome this issue but is primarily efficient at targeting proteins on the cell surface, whereas most therapeutic target molecules are likely to reside inside the cell.[154,155] Furthermore, aptamers may bind nonspecifically to dead cells that result during selection steps, potentially giving false-positive results and compromising the selection process.[155–157] However, fluorescence-activated cell sorting (FACS) and microbead-based methods to remove dead cells may help mitigate this issue.[158] Animal-based SELEX screening is inherently complicated, slow-paced, costly, and may need to be tailored for each animal model.[159] Therefore, the development of novel screening systems that work in a native biological context while still performing in an efficient and high-throughput manner is highly desirable.

Recently, in our lab, we developed a novel CRISPR-based RNA aptamer screening platform called "CRISmers." Using this newly developed technique, we successfully identified RNA aptamers specifically targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein, displaying both sensitive binding and neutralizing activity against the live SARS-CoV-2 virus and its Delta and Omicron variants. Furthermore, the intranasal delivery of an aptamer, modified with 2′-fluoro pyrimidines (2′-F), 2′-O-methyl purines (2′-O), and coupled with both cholesterol and polyethylene glycol of 40 kDa (PEG40K), demonstrated successful prophylactic and therapeutic antiviral efficacy against live Omicron BA.2 variants *in vivo*.[160] The CRISmers system offers advantages in screening RNA aptamers within cells, presenting a more natural environment for protein-RNA interactions. It is less susceptible to experimental conditions and background noise compared with solution-based methods.

Chemical modifications in RNA molecules

RNA molecules are notoriously unstable in the native cellular environment and are rapidly degraded by nucleases present in cells, as they are recognized as foreign entities. Several factors contribute to the degradation of RNA molecules in cells, including the presence of ribonucleases, which catalyze the hydrolysis of RNA, and the reactive nature of RNA's 2′-OH group. This instability presents a significant challenge for the development of RNA-based therapeutics. To address these challenges, successful efforts have been made to introduce a variety of chemical modifications that can increase the stability of RNA molecules in cells. These modifications include alterations to the sugar-phosphate backbone, the introduction of nucleotide modifications, and the use of modified bases.

Chemical modifications in ASOs

Modifications to the PS backbone were first introduced in ASOs in the 1980s and later in siRNAs, constituting the first generation of NA therapeutics.[161] In 1988, Stein et al. introduced phosphorothioate oligodeoxynucleotides (PS-ODNs) that contained either all-PS duplexes, duplexes with one normal chain and one all-PS chain, or were end-capped with several PS groups at both the 3′ and 5′ ends. The PS linkage is created by replacing a non-bridging oxygen with a sulfur atom, which increases resistance to nuclease degradation.[162–164] This PS modification alters the charge and conformation of the RNA molecule, making it less susceptible to degradation by

nucleases. Specifically, the PS modification results in a change in the local charge distribution of the RNA backbone, making it less negatively charged and therefore, less attractive to positively charged nucleases. Additionally, the PS modification can alter the structure and stability of the RNA molecule, leading to improved resistance to nuclease degradation. The sulfur atom in the PS linkage can form hydrogen bonds with neighboring atoms, leading to changes in the local structure of the RNA molecule that can stabilize it against nucleolytic degradation.[165] This modification significantly improved the stability of oligonucleotides in cells and tissues, protecting them from endogenous nucleases.[164] One example of this minimal chemical modification is Fomivirsen, the first approved ASO drug, which replaced oxygen atoms with sulfur atoms in the phosphodiester linkages of the nucleotide backbone. The use of a PS backbone substantially improved resistance to nuclease activity and increased binding to serum proteins, which in turn increased the half-lives of ASOs in serum,[166,167] while allowing the molecules to be used in applications that involve downregulation of target RNAs.[168] However, the increased binding of first-generation ASOs to serum proteins, while enhancing stability, also posed challenges in efficiently reaching target RNAs and raised concerns about potential toxicity.[169,170]

The improved stability from backbone modification encouraged researchers to experiment with the chemistry of other moieties of ASOs, such as the ribose base, leading to the development of less toxic second-generation ASOs. Modifications at the 2′ position of the ribose, including 2′-O-methyl (2′-OMe), 2′-O-aminopropyl (2′-O-AP), 2′-O-methoxy-ethyl (2′-MOE), and 2′-fluoro, have improved the safety and efficacy profiles of these molecules. The 2′-O-modification stabilizes RNA molecules by reducing their susceptibility to degradation by nucleases, thereby enhancing their stability and prolonging their half-life *in vivo*. Another proposed mechanism is that the 2′-O-modification enhances the binding affinity of RNA molecules to their target molecules, such as mRNA or miRNA, by increasing the flexibility and conformational diversity of RNA molecules. This can improve their ability to interact with target molecules and reduce off-target effects.[171] Intriguingly, these modifications result in the inability to recruit RNase H1 to the ASO-RNA duplex. However, their antisense effect can still be carried out by steric blockage of the translation initiation site.[12,172] Furthermore, second-generation ASOs that do not recruit RNase H1 can also be used to control alternative splicing and could play a role in the treatment of genetic diseases. For instance, phosphorothioate 2′-O-methyl-oligoribonucleotides were used to target the aberrant splice site to correct splicing of beta-globin mRNA.[173]

In analogy with the first two generations, third-generation ASOs showed higher resistance to degradation by nucleases and peptidases, as well as stronger affinities to target mRNAs. These desirable attributes of third-generation ASOs are due to modifications in the furanose ring, along with the aforementioned modifications of phosphate linkages or riboses and nucleotides. A variety of chemical modifications, including LNA, PNA, and morpholino phosphoroamidates (MF), are the three most commonly used third-generation ASOs. LNA is a bicyclic RNA analog in which the ribose ring is constrained in a C3′-endo conformation by a methylene bridge between the 2′-oxygen and 4′-carbon atoms. This modification increases the binding affinity of RNA molecules to their target molecules and reduces their susceptibility to degradation by nucleases.[174,175] PNAs are synthetic NA analogs featuring a peptide-like backbone, allowing them to hybridize with RNA sequences with high affinity and specificity. PNAs exhibit enhanced stability, resistance to nucleases, and reduced immune activation, making them a promising tool for targeting RNA molecules. For instance, PNA-mediated inhibition of miRNA has been explored with long-lasting effects and no cytotoxicity.[176] Additionally, PNA-based antisense oligomers have been utilized for the modulation of RNA splicing and targeting disease-causing RNA sequences in Duchenne muscular dystrophy.[177] In MF chemical modification, a morpholine ring is used instead of a ribose sugar as the backbone of the oligonucleotide. The morpholino backbone confers enhanced stability, as it does not show electrostatic interactions with other proteins, reduced off-target effects due to binding with a sequence of approximately 14–15 consecutive bases, and resistance to nucleases.[178] This evolution of chemical modifications has expanded the potential of ASOs for therapeutic applications, particularly in genetic disorders and viral infections.[179]

These modifications can be used independently or in combination to fully harness the advantages of chemical interactions within the cellular environment (Figure 2). A fascinating evolution of ASOs is the development of "gapmers," where a central DNA-oligo "gap" is surrounded by chemically modified RNA-oligo flanking regions that promote target binding.[180] The central DNA oligos retain RNase H1 competency, while the flanking modified RNA oligos provide stability and target binding. Gapmers offer several advantages over traditional ASOs, including increased specificity and potency, as well as improved stability and reduced off-target effects. They have shown promise as a therapeutic approach for a range of diseases, including genetic disorders, viral infections, and cancer.[181]

Chemical modification of siRNA

Negatively charged siRNAs are confronted by a combination of native multi-defense systems, including extracellular toll-like receptors (TLR-3, -7, -8) and intracellular sensors such as retinoic acid inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5).[182] However, chemical modifications, such as the 2′ addition of fluoro (F) and methoxy (OMe) groups, are highly tolerated by Argonaute proteins.

Modifications of the functional domain in the base sequence primarily include alterations in hydrogen bonding, electrostatic interactions, complexation, and generalized acid-base interactions. Specific base modifications include hydroxymethylation, guanine oxidation, carboxyl substitution, cytosine methylation, deamination, and adenine methylation.[183] Nucleobases are modified less frequently than the sugar-phosphate backbone due to their significant role in maintaining structural stability. Altered hydrogen bonding may cause the loss of base pairing between strands, preventing the formation of

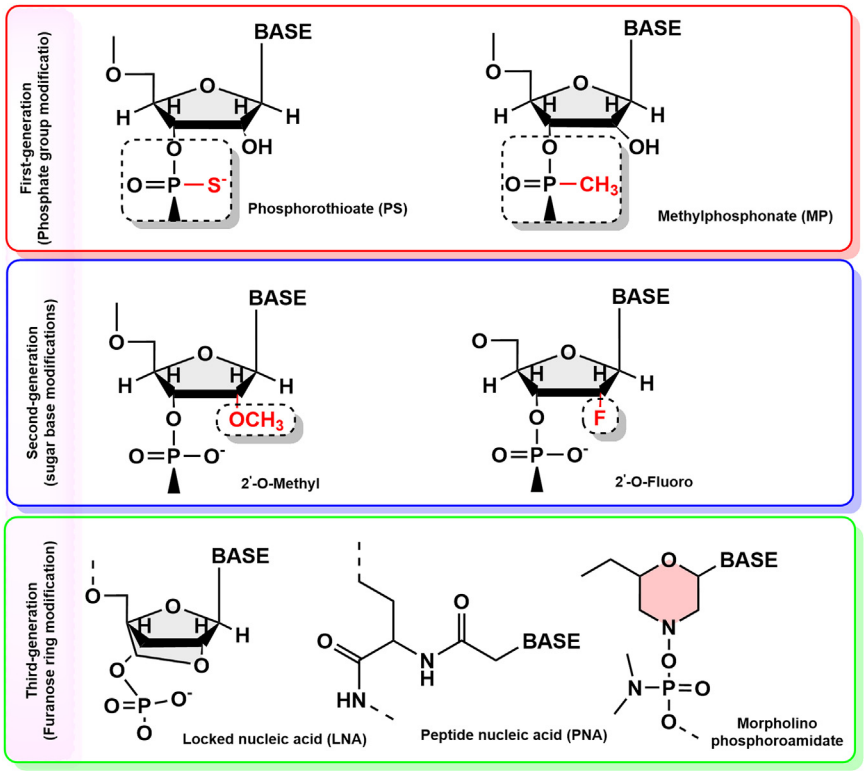

Figure 2. Evolution of nucleic acid modifications across generations

(Top) First generation: Phosphate group modifications with phosphorothioate (PS) and methylphosphonate (MP). (Middle) Second generation: Sugar modifications including 2′-O-Methyl and 2′-O-Fluoro for enhanced nuclease resistance. (Bottom) Third generation: Structural innovations like locked nucleic acid (LNA), peptide nucleic acid (PNA), and morpholino phosphoroamidate, optimizing specificity and stability.

certain base combinations. Despite this, base modifications can provide insights into the mechanism of gene silencing and lead to novel methods for overcoming off-target effects that arise from deleterious protein binding or mistargeting of mRNA.[184] Therefore, specific functions can be induced when designed nucleobase analogs are introduced into oligonucleotides (ONs), and even subtle changes, such as size or electronic distribution, can have a dramatic effect.

Backbone modifications, including methylphosphonate, PS, and boranophosphate (BP), which can be broadly classified as neutral, anionic, or cationic internucleoside linkages, are designed to overcome the physical and biological limitations of the natural phosphodiester linkage.[185,186]

Diastereomeric PS linkages are created when sulfur replaces the nonbridging oxygen atom. PS-ONs are the primary backbone modifications used successfully in gene silencing. PS linkages confer significant resistance to nuclease degradation, leading to higher bioavailability of ONs. In addition to nuclease resistance, PS-ONs also show improved absorption, distribution, and excretion profiles. It has been reported that PS modification can enhance binding affinity with plasma proteins, allowing PS-ONs to be rapidly absorbed from the injection site into the bloodstream,[187,188] These molecules exhibit good uptake in several tissues, including the kidney, liver, spleen, lymph nodes, adipocytes, and bone marrow, though not in skeletal muscle or the brain. Once they arrive at the target organ, PS-ONs can be quite stable due to chemical modifications, with a half-life of 1–4 weeks. However, their binding affinity to target ON sequences and specificity are less satisfactory, as PS-modified molecules exhibit no miRNA inhibitory activity.[189] Therefore, selective substitution of phosphodiester bonds with PS bonds is optimal for increasing nuclease resistance while retaining the ability to bind target miRNAs. Nevertheless, PS-ONs have increased toxicity, which is particularly problematic in siRNAs, where more than half of the phosphodiester bonds have been replaced with PS bonds.[190] Several studies have also reported that this type of modification impairs RISC activity, making it less favorable for use.[191] Another efficient modification is the use of BP, which increases lipophilicity compared with PS analogs and offers twice the resistance to nuclease degradation while causing low toxicity. However, BPs are not tolerated in the center of the guide strand, limiting their application.[192] A summary of chemical modifications has been added in Table 2.

Delivery of NA therapeutics

In contrast to the convenience of oral administration, as is the case with small molecules, NA drugs present a significant challenge in delivery to targeted sites due to their relatively larger sizes, high electric charges, and the inevitable encounter with endogenous nuclease systems. To enhance the delivery of NA drugs to specific target tissues, various techniques are employed, which can be broadly categorized into viral-based and non-viral-based delivery systems. In this section, we will discuss various NA drug delivery techniques.

Viral-based delivery systems

Viral-based delivery of NA therapeutics utilizes genetically modified viral vectors to deliver NA molecules into target cells. The most commonly used viral vectors for NA delivery are lentiviruses, retroviruses, and adeno-associated viruses (AAVs). AAV vectors are favored due to their low immunogenicity, ability to transduce both dividing and non-dividing cells, and long-term gene expression.[193] According to Nathwani and colleagues, AAVs have demonstrated long-term safety and efficacy in nonhuman primates and in gene therapy clinical trials of various diseases.[194,195] In another study, Pasi and colleagues are investigating the potential of AAV5-hFVIII-SQ therapy to treat

Table 2. List of chemical modifications used in NA therapeutics

Name	Modification profile	Advantages	Limitations	Applications
2′-O-Methylation (2′-OMe)	Addition of a methyl group at the 2′-hydroxyl of ribose	Increases stability, reduces immune recognition	May affect RNA function in some contexts	siRNA, ASOs
2′-Fluoro (2′-F)	Replacement of 2′-hydroxyl with fluorine	Enhances resistance to nucleases, increases binding affinity	Can increase the cost of synthesis	siRNA, ASOs
Locked Nucleic Acid (LNA)	Methylene bridge between 2′-oxygen and 4′-carbon	Increases binding affinity to complementary RNA strands	Increases synthesis complexity and cost	ASOs
5-Methylcytosine (5mC)	Methylation at the 5th carbon of cytosine	Enhances base pairing stability, reduces immune activation	May not be effective in all contexts	ASOs, mRNA
Phosphorothioate (PS) Linkages	Replacement of non-bridging oxygen with sulfur in the phosphate backbone	Increases nuclease resistance, prolongs half-life	Can introduce toxicity and off-target effects	siRNA, ASOs
Phosphorodiamidate Morpholino Oligomers (PMOs)	Replacement of ribose with morpholine ring and phosphorodiamidate linkage	High stability, resistance to enzymatic degradation	Limited to specific applications	ASOs
2′-O-Methoxyethyl (2′-MOE)	Addition of methoxyethyl group at the 2′ position	Enhances stability, reduces immune stimulation	Can introduce off-target effects	siRNA, ASOs
Anti-Reverse Cap Analog (ARCA)	Modified cap structure preventing reverse attachment	Ensures proper translation and stability	Specific to mRNA applications	mRNA therapeutics, vaccines
Cap 1 and Cap 2 Structures	Additional methylations at first and second nucleotides adjacent to the cap	Enhances stability, reduces immune activation	Specific to mRNA applications	mRNA therapeutics, vaccines
Pseudouridine (Ψ)	Isomerization of uridine	Increases stability, reduces immune response	Not universally accepted by all cellular machinery	mRNA vaccines, therapeutics
N1-Methylpseudouridine (m1Ψ)	Methylation at N1 position of pseudouridine	Further increases stability, reduces immunogenicity	Limited understanding of long-term effects	mRNA vaccines, therapeutics
GalNAc Conjugation	Conjugation with N-Acetylgalactosamine	Facilitates liver-specific delivery	Specific to hepatocyte targeting	siRNA, ASOs
PEGylation	Attachment of polyethylene glycol (PEG)	Increases solubility, reduces clearance, decreases immunogenicity	May cause immune reactions, complicates manufacturing	siRNA, ASOs

severe hemophilia. A 3-year follow-up of the clinical trial revealed a decrease in bleeding events and the complete cessation of prophylactic factor VIII therapy.[196] Using a viral vector approach in therapeutics involves inserting the NA molecule into the viral vector, which can then transduce target cells and deliver the NA to the nucleus. Once inside the target cell, the NA therapeutic can function to degrade specific mRNA molecules or introduce protein-coding sequences, depending on its intended mechanism of action. Viral vector-based delivery systems offer a promising approach for developing effective treatments for various diseases.

In recent years, gene therapies utilizing viral AAV as vector delivery systems have demonstrated promising results in treating various genetic disorders. Currently, there are over 200 ongoing clinical trials employing AAV vectors, which highlights the significant research and therapeutic potential in this field.[197] Moreover, a number of gene therapies have received FDA approval that employ AAV vectors. In this regard, Luxturna (voretigene neparvovec-rzyl) was the first AAV-gene therapy that received FDA approval in late 2017. It is designed to treat a rare inherited retinal dystrophy caused by mutations in the *RPE65* gene by delivering a functional copy of the *RPE65* gene directly to the retina.[198,199] Hemgenix (etranacogene dezaparvovec) is another AAV-based gene therapy that received FDA approval in 2022 as the first gene therapy for hemophilia B. This therapy uses an AAV5 vector to deliver a working copy of the *F9* gene, which encodes coagulation factor IX, reducing the need for regular factor IX infusions. Clinical trials demonstrated a significant reduction in bleeding episodes and increased factor IX activity levels, improving the quality of life for patients.[200]

Casgevy (Exagamglogene autotemcel, or exa-cel), a cell-based gene therapy, marks a significant milestone as the first FDA-approved treatment employing CRISPR-Cas9 genome editing to modify hematopoietic stem cells in patients aged 12 years and older.[201] Exa-cel is engineered to induce fetal hemoglobin production through *ex vivo* CRISPR-Cas9 editing of autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) at the BCL11A erythroid-specific enhancer. In a phase 3, single-group, open-label clinical trial, patients aged 12 to 35 with sickle cell disease and a history of severe vaso-occlusive crises were treated. The trial involved editing CD34+ HSPCs using

Table 3. List of selective NA therapeutics in clinical trials (up to August 2024)

RNA type	Current status	Drug name(s)	Target profile	Chemical modification and carrier	Developers	Administration
ASO	Phase 1	NCT05018533	Broad indication (healthy volunteer)	–	TAKC-02 (TAK-Circulator Co.)	Inhalation
		NCT04504669	Cancer (clear cell renal cell cancer, non-small-cell lung cancer, triple-negative breast neoplasms, squamous cell cancer of head and neck, small cell lung cancer, gastroesophageal cancer, melanoma, cervical cancer, advanced solid tumors)	Mod/Subs (cEt)	AZD8701 (AstraZeneca)	i.v
		NCT04072458	Cancer (mantle cell lymphoma, peripheral T-cell lymphoma [PTCL], cutaneous T-cell lymphoma [CTCL], chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), follicular lymphoma, marginal zone lymphoma, Hodgkin lymphoma, waldenstrom macroglobulinemia, diffuse large B-cell lymphoma)	Liposome	L-Bcl-2 antisense oligonucleotide (Bio-Path Holdings)	i.v
		NCT04539041	Physiological (progressive supranuclear palsy)	Other (unspecified)	Unnamed ASO (Novartis)	i.t
	Phase 1/2	NCT05032196	Genetic (Huntington disease)	Mod/Subs (PS)	WVE-003 (Wave Life Sciences)	i.t
		NCT04906460	Genetic (Duchenne muscular dystrophy)	Mod/Subs (PS)	WVE-N531 (Wave Life Sciences)	i.v
		NCT04931862	Physiological (amyotrophic lateral sclerosis, frontotemporal dementia)	Mod/Subs (PS)	WVE-004 (Wave Life Sciences)	i.t
		NCT03780257	Genetic (retinitis pigmentosa, Usher syndrome type 2, deaf blind, retinal disease, eye diseases, vision disorders)	Mod/Subs (PS-2′-OMe)	QR-421a (ProQR Therapeutics)	i.v.t
	Phase 2	NCT02781883	Cancer (acute myeloid leukemia)	Liposome	BP1001 (Bio-Path Holdings)	i.v
		NCT04740476	Genetic (Dravet syndrome)	Mod/Subs (PS-2′-MOE)	STK-001 (Stoke Therapeutics)	i.t
		NCT03702829	Physiological (amyloidosis)	Mod/Subs (2′-MOE)	Inotersen (Brigham and Women's Hospital)	s.c
	Phase 2/3	NCT03913143	Genetic (Leber congenital amaurosis 10)	Mod/Subs (PS-2′-OMe)	Sepofarsen (ProQR Therapeutics)	i.v.t
siRNA	Phase 1	NCT03772249	Infectious (hepatitis B, chronic)	Conjugate (GalNAc)	DCR-HBVS (Dicerna Pharmaceuticals)	s.c
		NCT01591356	Cancer (advanced malignant solid neoplasm)	Liposome	EphA2-targeting DOPC-encapsulated siRNA (National Cancer Institute)	i.v
		NCT03819387	Cancer (non-small cell lung cancer, pancreatic cancer, colorectal cancer)	Lipid nanoparticle (LNP)	NBF-006 (Nitto BioPharma)	i.v
		NCT04995536	Cancer (recurrent B-cell non-Hodgkin lymphoma)	Conjugate (CpG oligodeoxynucleotide)	CpG-STAT3 siRNA (National Cancer Institute)	i.v
	Phase 1/2	NCT02949830	Genetic (acute intermittent porphyria)	Conjugate (GalNAc)	Givosiran (Alnylam Pharmaceuticals)	s.c
	Phase 2	NCT01676259	Cancer (pancreatic ductal adenocarcinoma, pancreatic cancer)	Other	siG12D-LODER (Silenseed Ltd)	Implant
		NCT04844840	Cosmetic (Keloid)	Other (Non-lipid NP)	STP705 (Sirnaomics)	s.c
		NCT04666298	Genetic (hypercholesterolemia, heterozygous familial hypercholesterolemia)	Conjugate (GalNAc)	Inclisiran (Novartis)	s.c

(*Continued on next page*)

Table 3. *Continued*

RNA type	Current status	Drug name(s)	Target profile	Chemical modification and carrier	Developers	Administration
	Phase 3	NCT03759379	Genetic (amyloidosis, hereditary, transthyretin amyloidosis)	Conjugate (GalNAc)	Patisiran and Vutrisiran (Alnylam Pharmaceuticals)	s.c
		NCT04042402	Genetic (primary hyperoxaluria type 1, primary hyperoxaluria type 2, kidney diseases, urologic diseases, genetic disease)	Conjugate (GalNAc)	DCR-PHXC (Dicerna Pharmaceuticals)	s.c
		NCT04153149	Genetic (transthyretin amyloidosis [ATTR] with cardiomyopathy)	Conjugate (GalNAc)	Vutrisiran (Alnylam Pharmaceuticals)	s.c
		NCT03681184	Genetic (primary hyperoxaluria type 1)	Conjugate (GalNAc)	Lumasiran (Alnylam Pharmaceuticals)	s.c
		NCT03814187	Genetic (atherosclerotic cardiovascular disease, elevated cholesterol, heterozygous familial hypercholesterolemia, homozygous familial hypercholesterolemia)	Conjugate (GalNAc)	Inclisiran (Novartis)	s.c
		NCT04819269	Inflammation (dry eye disease, Sjogren syndrome)	Other (Naked)	Tivanisiran (Sylentis)	Ocular
Aptamers	Phase 1	NCT05018403	Other (Healthy)	Conjugate (PEG)	AON-D21 (Aptarion Biotech)	i.v
	Phase 2	NCT04677803	Genetic (Von Willebrand diseases, hemophilia A)	Conjugate (PEG)	BT200 (Medical University of Vienna)	s.c
	Phase 4	NCT02321267	Physiological (macular diseases)	Conjugate (PEG)	Pegaptanib (Kagawa University)	i.v.t
mRNA	Early Phase 1	NCT02872025	Cancer (carcinoma, intraductal, noninfiltrating)	LNP	mRNA 2752 (Merck Sharp & Dohme Corp., Moderna)	Intratumoral
	Phase 1	NCT04573140	Cancer (adult glioblastoma)	Liposome	mRNA-loaded DOTAP liposome (CureSearch)	i.v
		NCT04916431	Inflammation (various autoimmune disorders)	LNP	mRNA-6231 (Moderna)	s.c
		NCT02316457	Cancer (triple-negative breast cancer [TNBC])	Liposome	IVAC_W_bre1_uID/IVAC_M_uID (BioNTech)	i.v
		NCT04442347	Genetic (ornithine transcarbamylase deficiency)	LNP	ARCT-810 (Arcturus Therapeutics)	i.v
		NCT05001373	Infectiious (HIV)	LNP	Core-g28v2 60mer and eOD-GT8 60mer (Moderna)	i.m
		NCT04144348	Infectious (human metapneumovirus and human parainfluenza)	LNP	mRNA-1653 (Moderna)	i.m
		NCT03713086	Infectious (rabies)	LNP	CV7202 (CureVac)	i.m
		NCT04528719	Infectious (respiratory syncytial virus)	LNP	mRNA-1345 (Moderna)	i.m
		NCT03871348	Cancer (metastatic neoplasm)	LNP	SAR441000 (Sanofi, BioNTech)	Intratumoral
		NCT03948763	Cancer (neoplasms, carcinoma, non-small-cell lung cancer, pancreatic neoplasms, colorectal neoplasms)	LNP	V941 (Merck Sharp & Dohme Corp.)	i.m
		NCT04163094	Cancer (ovarian cancer)	Liposome	W_ova1 Vaccine (BioNTech)	i.v
		NCT03313778	Cancer (solid tumors)	LNP	mRNA-4157 (Moderna, Merck Sharp & Dohme Corp.)	i.m
		NCT05043181	Genetic (familial hypercholesterolemia)	Other (Exosome)	Low-Density Lipoprotein Receptor mRNA Exosomes (Tang-Du Hospital, Air Force Military Medical University, China)	i.v

(*Continued on next page*)

Table 3. ***Continued***

RNA type	Current status	Drug name(s)	Target profile	Chemical modification and carrier	Developers	Administration
	Phase 1/2	NCT04956575	Infectious (seasonal influenza)	LNP	mRNA-1010 (Moderna)	i.m
		NCT03164772	Cancer (Metastatic non-small cell lung cancer)	LNP	BI 1361849 (MedImmune, CureVac, PharmaJet)	Intradermal
		NCT04990388	Genetic (glycogen storage disease type III)	LNP	UX053 (Ultragenyx Pharmaceutical)	i.v
		NCT05130437	Genetic (propionic acidemia)	LNP	mRNA-3927 (Moderna)	i.v
	Phase 2	NCT04917861	Infectious (Zika virus)	LNP	mRNA-1893 (Moderna)	i.m
		NCT04534205	Cancer (unresectable head and neck squamous cell carcinoma, metastatic head and neck cancer, recurrent head and neck cancer)	Liposome	BNT113 (BioNTech)	i.v
		NCT03897881	Cancer (melanoma)	LNP	mRNA-4157 (Moderna, Merck Sharp & Dohme Corp.)	i.m
	Phase 2/3	NCT04652102	Infectious (COVID-19)	LNP	CVnCoV (CureVac)	i.m
	Phase 3	NCT05085366	Infectious (cytomegalovirus)	LNP	mRNA-1647 (Moderna)	i.m
CRISPR-Cas	Phase 1	NTLA-2002	Hereditary angioedema	LNPs	Intellia Therapeutics	i.v
		NTLA-2001	ATTR amyloidosis with cardiomyopathy	LNPs	Intellia Therapeutics, Regeneron	i.v
	Phase 1/2	NTLA-2001	Transthyretin amyloidosis	LNPs	Intellia Therapeutics, Regeneron	i.v
		EDIT-301	Sickle cell disease	LNPs	Editas Medicine	i.v
		Exagamglogene autotemcel (exa-cel)	Sickle cell disease, Beta-thalassemia	Lentiviral vector	CRISPR Therapeutics, Vertex Pharmaceuticals	i.v
		BEAM-302	Alpha-1 antitrypsin deficiency	LNPs	Beam Therapeutics	i.v
		CB-010	Large B cell lymphoma	Donor-derived CAR-T cells	Caribou Biosciences	i.v
		UCART22	Relapsed/refractory acute lymphoblastic leukemia (ALL)	Allogeneic CAR-T cells	Cellectis	i.v
		Locus Biosciences CRISPR-Cas3 therapy	Chronic urinary tract infections (UTIs)	Bacteriophages with CRISPR-Cas3	Locus Biosciences	i.v
		RENI-CEL (formerly EDIT-301)	Sickle cell disease, Beta-thalassemia	CRISPR-Cas12a ribonucleoprotein	Editas Medicine	i.v
–	Phase 2	NCT06500273	Large B cell lymphoma (LBCL)	CRISPR-Cas12a ribonucleoprotein	Allogene Therapeutics	i.v

CRISPR-Cas9, followed by myeloablative conditioning with busulfan before exa-cel infusion. The primary endpoint was the absence of severe vaso-occlusive crises for at least 12 consecutive months. Results showed that of the 30 patients with adequate follow-up, 29 (97%) were free from vaso-occlusive crises, and all 30 (100%) avoided hospitalizations for severe crises for at least 12 months. The safety profile of exa-cel was consistent with expectations for myeloablative busulfan conditioning and autologous HSPC transplantation, with no incidences of cancer reported. This study demonstrates that exa-cel can effectively eliminate vaso-occlusive crises in a significant majority of patients with SCD for 12 months or longer.[202]

Despite their promise, viral-based delivery systems also present challenges. For instance, some patients have pre-existing immunity due to prior exposure to wild-type viruses, which poses a significant challenge for infused viral vectors.[203,204] Moreover, the use of viruses in viral-based drugs can cause immunogenicity and provoke immune responses that could interfere with the therapeutic transgene products and other signaling pathways, potentially leading to treatment failure.[205,206] The cargo size of AAVs, the most widely used viral vector in therapeutics, is about 4.7 kb. While there have been attempts to increase the cargo size, studies have shown that the efficiency of AAV systems is compromised with cargos over 5 kb.[207] Genomic integration of viral sequences is another concern of viral-based drug systems. A study extended to 4 years using AAV vectors in dogs revealed 1,741 AAV integration events in the genomic DNA of the host.[208]

Non-viral-based delivery systems

Nanoparticles possess unique physicochemical properties that make them ideal for protecting NA molecules from native nucleases and facilitating safe delivery to target tissues. However, directing nanoparticles to specific target sites remains a significant challenge in NA delivery. Various strategies have been developed to improve the efficiency of nanoparticle-based NA delivery, as reviewed in the literature.[209] The quest to identify the most effective nanoparticles for NA encapsulation has progressed significantly, with several classes of nanoparticles being investigated.

Polymers and polymeric nanoparticles have been extensively used for delivering NA molecules to target sites.[210] The physical and chemical properties of polymers and polymeric nanoparticles can be fine-tuned to enhance the delivery of desired NAs to specific target locations within cells.[211,212] An FDA-approved molecule, poly(lactic-co-glycolic acid) (PLGA), which lacks a positive charge to complex with NA moieties,[213] has traditionally been used for delivering small molecules. However, PLGA has been modified by adding cationic chemical groups, such as chitosan, to deliver siRNA in mice.[214] Some polymeric nanoparticles inherently contain cationic groups, such as poly(L-lysine) (PLL) and polyethylenimine (PEI), enabling them to electrostatically bind NA molecules. However, the high molecular weight (MW) of PLL and PEI poses severe toxicity risks due to the higher cationic charge, which can lead to cell surface, mitochondrial, and nuclear membrane disruptions, ultimately causing apoptosis,[215] endosomal escape issues,[216] non-specific protein binding,[217] and immune stimulation.[218] Various modifications have been explored to reduce the MW of these particles and increase their efficiency. For example, disulfide cross-linked low-molecular-weight linear PEIs have demonstrated low toxicity and high efficiency in multiple cell lines.[219] This success encouraged further modifications, such as PEG-grafted PEIs, which were successfully used for delivering mRNA to lung cells in mice.[220] Another example includes the synthesis of β-cyclodextrin (β-CD) and branched PEI conjugates for delivering an mRNA vaccine. These conjugates encapsulate mRNA, allowing it to pass through plasma membranes and escape endosomal degradation, resulting in increased transfection efficiency.[221]

Lipid nanoparticles (LNPs) have emerged as a prevalent choice for encapsulating NA therapeutics due to their biocompatibility, versatility, and high transfection efficiency. LNPs have already been utilized in FDA-approved NA therapeutics, such as patisiran and inclisiran, and are being investigated for many other NA therapeutics currently in clinical trials. The success of LNPs in NA delivery highlights the potential of nanoparticle-based delivery systems in the future of molecular medicine.[222] LNPs consist of four distinct components: a cationic or ionizable lipid, cholesterol, a helper lipid, and a poly(ethylene glycol) (PEG) lipid. Depending on the sizes of their hydrophilic head and hydrophobic tail, lipid-based nanoparticles can form various structures, including micelles, liposomes, and LNPs (Figures 3A–3C).[29] Several LNPs, including epoxide-amine-based C12-200,[223] the peptide-like lipid called cKK- E12,[224] DLin- KC2-DMA (an ionizable lipid identified using rational design), and DLin-MC3-DMA123,[225,226] have been successfully designed for siRNA delivery to nonhuman primates. Beyond siRNA delivery, LNPs have recently been employed to deliver mRNAs as well.[29] For example, in a recent study, *in vivo* production of anti-fibrotic CAR-T cells was achieved by packaging modified mRNAs in decorated LNPs with guiding antibodies capable of producing CAR in T-lymphocytes.[227] However, several challenges remain in utilizing LNPs, including the complex synthesis process and the potential for the molecule itself to prompt an immune response.[228] Additionally, liver accumulation *in vivo* represents a significant barrier in developing therapeutically efficacious nanoparticle drug delivery systems.

For efficient siRNA delivery, N-acetylgalactosamine (GalNAc) conjugates have emerged as a vital breakthrough and a promising tool for NA delivery. Their tissue specificity, high transfection efficiency, biocompatibility, and stability make them a suitable choice for developing NA therapeutics for liver diseases. The success of GalNAc-siRNA conjugates is the result of half a century of research in targeted drug design and delivery. The asialoglycoprotein receptor (ASGPR), also known as the Ashwell-Morell receptor, is predominantly expressed on the surface of hepatocytes and removes glycoproteins with exposed terminal galactose and GalNAc glycans from circulation via clathrin-mediated endocytosis (Figure 3D). These receptors were first identified in the early 1970s, contributing significantly to the advancements in drug delivery.[229,230] In the following years, the successful targeted delivery of various molecules to the liver by exploiting ASGPR ligands paved an encouraging path for efficient targeted

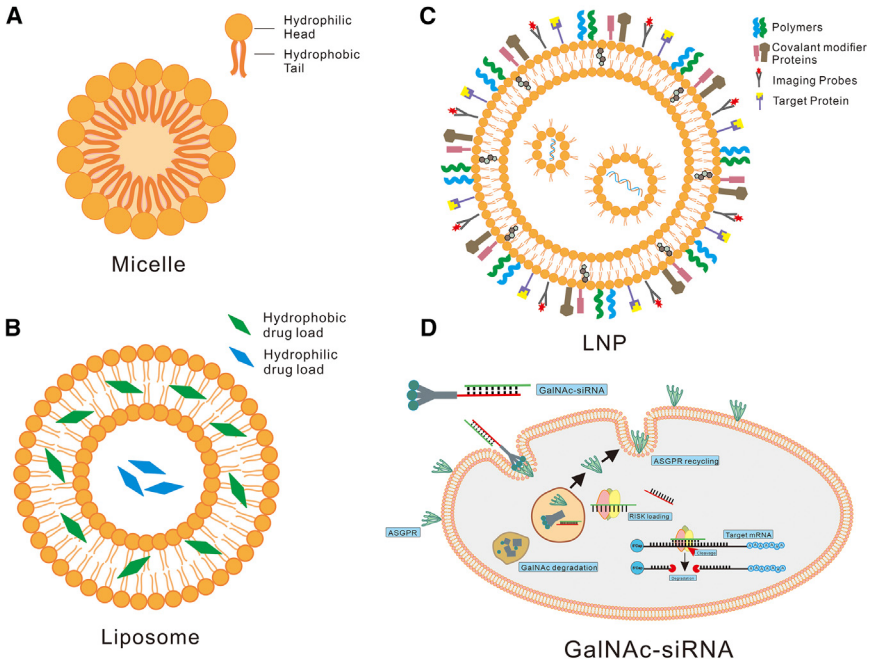

Figure 3. Schematic illustrations of different nanoparticle-based delivery systems

(A) Micelle, used for encapsulating hydrophobic drugs. (B) Liposome, capable of carrying both hydrophobic and hydrophilic drugs. (C) Lipid nanoparticle (LNP), incorporating various functional components for targeted drug delivery. (D) GalNAc-siRNA conjugate, facilitating targeted siRNA delivery to hepatocytes via the ASGPR pathway.

delivery.[32] For instance, the delivery of asialofetuin-linked antivirals trifluorothymidine and adenine-9-β-D-arabinofuranoside (ARAA) and adenine-9-β-arabinofuranoside 5′-monophosphate (ARA-AMP) resulted in a 3-fold reduction of hepatic Ectromelia viral DNA replication *in vivo*.[231,232] Further studies demonstrated that targeting ASGPR is capable of delivering diverse cargo and eliciting an array of biological responses *in vivo*, such as low-density lipoprotein (LDL) and diphtheria toxin.[233] In the decades following their discovery, the ASGPR and its ligands, including galactose and galactose derivatives like GalNAc, have emerged as promising delivery vehicles for a wide range of therapeutic molecules. These ligands have been used to deliver biologically active molecules such as glycopeptides, glycolipids, small molecules, nucleoside analogs, plasmid DNA, and ASOs to hepatocytes in the liver. The use of GalNAc conjugates to deliver siRNAs has gained particular attention as a robust and efficient method of mRNA suppression in the liver. GalNAc-siRNA conjugates are administered subcutaneously and are avidly taken up by the ASGPR expressed on the surface of liver cells. This specific targeting mechanism allows for highly targeted and potent silencing of genes of interest in the liver, making GalNAc conjugates a promising avenue for the treatment of a wide range of liver diseases.[234] Interdisciplinary research and progress in the above-mentioned aspects have seen encouraging progress. As a result, we have seen several approvals from the FDA and European Medicines Agency for NA therapeutics (Table 1) and can expect further acceleration to address medical challenges (Table 3).

NA THERAPEUTICS IN THE PAST 5 YEARS

The last 5 years have seen unprecedented progress in the development of NA therapeutics and their approvals for clinical use. Remarkably, out of 21 approved NA therapeutics, 13 NAs were approved during this period (Table 1), with several more expected to receive approval in the near future, currently undergoing clinical trials (Table 3).

The unprecedented COVID-19 pandemic, declared a global emergency by the World Health Organization (WHO) in 2019, affected millions globally, leading to extensive efforts to develop prevention and treatment strategies.[235,236] Although various drugs were tested, none were definitive, prompting a global push to create vaccines, including mRNA-based options. Existing knowledge of RNA therapeutics played a crucial role in the rapid development of mRNA COVID-19 vaccines, which were approved under emergency use and produced in record time. mRNA COVID-19 vaccines use the same basic principle as NA therapeutics: injecting antigen-encoding mRNA molecules encapsulated in a delivery vehicle, such as lipid nanoparticles (LNPs), into the body.[237] The mRNA is translated, producing antigen proteins that provoke an immune response. For instance, the first two mRNA COVID-19 vaccines approved, Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273), both encode the SARS-CoV-2 spike glycoprotein (S).[49,238] According to WHO data, the success rate of mRNA COVID-19 vaccines appears higher compared with other types of vaccines. Currently, over 300 COVID-19 vaccine candidates are under development, with at least 47 based on mRNA sequences. Interestingly, nearly half of these mRNA vaccines have already entered clinical trials.[239] The rapid development and the number of clinical trials reflect the therapeutic potential of NA molecules.

Another significant milestone during this period is the approval of the first CRISPR-Cas9 therapeutic. The groundbreaking gene editing technology was first introduced in 2012,[240] and the first CRISPR-Cas9 therapeutic, Casgevy, was approved for clinical use in 2023. This remarkable achievement, taking only about a decade from its initial discovery to clinical approval, may represent the shortest time for any molecule or therapeutic technology to receive approval after its discovery. The rapid advancement from bench to bedside for CRISPR therapeutics underscores the potential and efficiency of this gene editing technology. Clinical trials have demonstrated not only the efficacy but also the safety of these therapeutics, paving the way for future applications in various genetic disorders. This achievement highlights the importance of continued investment in

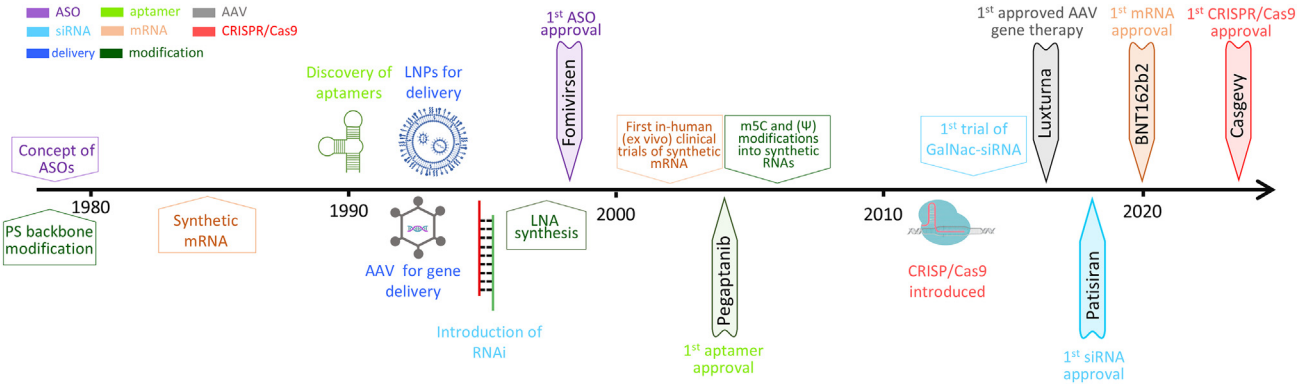

Figure 4. An overview of key milestones in RNA therapeutics
Showcasing significant chemical changes, delivery methods, and landmark drug approvals.

biotechnology research and development, regulatory agility, and interdisciplinary collaboration to bring innovative treatments to patients more swiftly.

The approval of Casgevy has catalyzed a surge in CRISPR-related research and development. Numerous clinical trials are now under way, exploring the use of this technology to treat a wide range of conditions, including cancers, blood disorders, and inherited genetic diseases. The approval of Casgevy has accelerated the CRISPR pipeline, with several therapies now in Phase II and III trials. Notably, Intellia Therapeutics' NTLA-2001 for amyloidosis and CRISPR Therapeutics' CTX-110 for oncological indications are promising candidates expected to reach the clinical stage soon (Table 3).[241,242] The technology's potential for treating various genetic disorders and cancers continues to drive innovation and investment in this transformative field.

In addition to CRISPR advancements, the FDA has approved several ASO therapies in the past 5 years, significantly accelerating the pace of NA therapeutic approvals and shaping the future of this field. Notable approvals include Golodirsen (Vyondys 53) in 2019, Viltolarsen (Viltepso) in 2020, Casimersen (Amondys 45) in 2021, and Wainua (eplontersen) in 2023 (Table 1). These therapies, primarily targeting Duchenne muscular dystrophy (DMD) by inducing exon skipping to restore dystrophin production, exemplify the rapid advancement and increasing efficiency in the regulatory approval process.

Similarly, over the past 5 years, the FDA has approved several siRNA therapeutics, reflecting a significant acceleration in the approval process and highlighting the growing impact of NA therapeutics. Notable approvals include givosiran (Givlaari) in 2019 for acute hepatic porphyria,[43] lumasiran (OXLUMO) in 2020 for primary hyperoxaluria type 1,[44] inclisiran (Leqvio) in 2020 for heterozygous familial hypercholesterolemia and clinical atherosclerotic cardiovascular disease,[45] and vutrisiran (Amvuttra) in 2022 for hereditary transthyretin-mediated amyloidosis.[243] Dicerna's nedosiran is the latest addition in the siRNA-approved family and used for treating the genetic disorder primary hyperoxaluria (PH), a family of ultra-rare, life-threatening genetic disorders that initially manifest with complications in the kidneys (Table 1).[243] These approvals underscore the rapid advancements in siRNA technology, particularly the use of GalNAc conjugates to enhance liver-specific delivery and improve therapeutic efficacy.

The success of NA therapeutics is the outcome of continuous progress and multidisciplinary success including conceptual breakthroughs, chemical modifications, improved delivery systems, and dedicated clinical trials (Figure 4). The surge in NA therapy approvals in past 5 years highlights the growing impact and potential of NA therapeutics to address previously untreatable genetic disorders, paving the way for future advancements in precision medicine and genetic disease treatment.

FUTURE PROSPECTS

The advantages of NA therapeutics over conventional modalities suggest a bright future for this field. We have witnessed significant progress in recent years, with several NA-based therapies receiving regulatory approvals and numerous others advancing through clinical trials. The rapid development and production of mRNA vaccines during the COVID-19 pandemic have provided a promising outlook for NA therapeutics on a global scale.[49] This unprecedented achievement in controlling the pandemic underscores the potential of NA therapeutics as a strategy to combat future threats.

The utility of mRNA therapeutics is rapidly expanding beyond COVID-19 vaccines. Recently, an open-label, randomized phase 2b trial of Moderna's personalized mRNA-based cancer vaccine, mRNA-4157, combined with the immunotherapy drug pembrolizumab (Keytruda), resulted in improved recurrence-free survival (RFS) in melanoma patients, with a 44% reduction in the risk of recurrence or death compared with pembrolizumab alone.[244] Additionally, a phase 2a trial of intracardiac injection of VEGF-A mRNA (AZD8601, Moderna) in hyperfused regions of patients undergoing heart surgery yielded significant results, offering hope for future cardiac regenerative therapies.[245]

mRNA-based *in vivo* CAR-T therapeutics is an emerging approach that offers unique advantages over the production of autologous CAR-T cells *in vitro*, which may suffer from batch variations and require significant time, potentially delaying treatment.[246,247] Recently, *in vivo* lipid nanoparticle (LNP)-mediated delivery of mRNA-encoded CRISPR has also shown early clinical success. AAV-mediated delivery of CRISPR can persist at the target site even after achieving the desired editing events, which may lead to off-target effects.[248] In contrast, the mRNA-encoded CRISPR approach allows mRNAs to remain active for a shorter period, achieving target edits before degradation by native enzymatic systems. This method has been successfully employed to knock down several disease-causing genes, showing potential for future mRNA therapeutics. For instance, reducible LNP-mediated delivery of CRISPR mRNAs to the liver targeting Angiopoietin-like 3 (Angptl3) resulted in a median editing rate of 38.5%, a corresponding 65.2% reduction of serum ANGPTL3 protein, and no off-target effects.[249] Similarly, the treatment of hereditary amyloidosis (ATTR) has also gained attention, with LNP-encapsulated CRISPR-mRNA and gRNA resulting in a significant reduction of circulating amyloid.[250] While most studies have targeted the liver for mRNA-based genome editing therapies, the next frontier lies in successfully targeting other organs and solid tissues such as the brain, kidneys, lungs, and heart, each posing unique challenges that must be overcome.

ASOs, being the earliest studied NA therapeutics with a simpler mechanism of action and multimodal gene expression modulation, have garnered the most FDA approvals in terms of number, from first-generation to second-generation chemical modifications (Table 1). In the last decade, several generation-2.5 ASOs containing 2' constrained ethyl (2'cEt) modifications have entered trial phases.[251] These trials include ARRx(AZD), targeting androgen receptor (AR) to treat castration-resistant prostate cancer (CRPC), and Custirsen (OGX-011), an inhibitor of CLU protein production, in combination with gemcitabine in patients with advanced non-small cell lung cancer (NSCLC).[252] Generation-2.5 ASOs contain a bicyclic sugar that constrains the furanose surrogate to adopt a conformation favorable to hybridization to RNA, increasing RNA affinity per nucleotide by approximately 2°C in thermal stability compared with their predecessors, PS 2'-MOE ASOs.[253] Additionally, the use of another 2' bicyclic sugar, LNA, provides even greater nucleotide affinity than the cEt modification, resulting in approximately 10-fold increased potency. However, this enhanced potency is accompanied by a notable rise in the incidence and severity of cytotoxicity.[254,255]

Recently, combinations of PS and chiral mesyl-phosphoramidate (MsPA) modifications have been employed in the quest for next-generation chemical modifications. Interestingly, site-specific replacement of PS linkages with MsPA has been shown to enhance potency while reducing non-specific protein binding and cytotoxicity.[256]

The first approved siRNA drug, patisiran, was approved by the FDA in 2018 for the treatment of hereditary transthyretin-mediated (hATTR) amyloidosis and is delivered using an LNP-based system.[226] However, the subsequent approvals of siRNA drugs, givosiran (targeting ALA synthase 1 (ALAS1) for acute hepatic porphyria) and inclisiran (targeting PCSK9 expression to reduce LDL cholesterol levels), utilized GalNAc conjugate-mediated delivery to hepatocytes. Notably, the GalNAc conjugate-mediated delivery represents a significant milestone in siRNA therapeutics, as it not only improves targeting but also provides a long-lasting effect for over 6 months.[257]

A number of siRNA drugs are currently in trials. For instance, Dicerna has developed siRNA-based nedosiran for treating the genetic disorder primary hyperoxaluria (PH), a family of ultra-rare, life-threatening genetic disorders that initially manifest with complications in the kidneys. Nedosiran inhibits the production of the hepatic lactate dehydrogenase (LDH) enzyme, involved in converting glyoxylate to oxalate. A multi-dose phase 3 open-label trial confirmed long-term safety and a reduction of urinary oxalate to near-normal and even normal ranges in PH1 and PH2 patients.[258] Nedosiran also uses GalNAc conjugate-mediated delivery for accurate targeting and efficient results and approved for clinical use in 2023.[46] However, there remains a need to develop delivery systems targeting other organs as well.

Overall, the advancement of NA therapeutics as a potentially major mode of therapy is intricately connected to progress in several key areas, such as enhancing stability in the native cellular environment, ensuring cost-effectiveness, and achieving efficient and specific delivery to the target site. Significant improvements in these aspects are crucial to fully realize the potential benefits of NA therapeutics in the future.

ACKNOWLEDGMENTS

We thank all colleagues in our laboratories for helpful discussion. This work was supported by the National Key Research and Development Program of China (2023YFA0915000 to Y.W.), National Natural Science Foundation of China (82273967 to Y.W.), National Natural Science Foundation of China (82273890 to Y.Z.), Department of Science and Technology of Guangdong Province (No. 2021QN020576 to Y.W.), Shenzhen Stable Support Grant (GXWD 20231130103401001 to Y.Z.),and Shenzhen Science and Technology Program (JCYJ20240813104817024 to Y.Z.), China Postdoctoral Science Foundation (No. 2023M742397 to J.Z.), Postdoctoral Fellowship Program of CPSF (No. GZC20231724 to J.Z.).

AUTHOR CONTRIBUTIONS

S.N. and J.Z. prepared the manuscript. Y.Z. and Y.W. provided funding and resources and participated in the writing and revision of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Piovesan, A., Caracausi, M., Antonaros, F., Pelleri, M.C., and Vitale, L. (2016). GeneBase 1.1: a tool to summarize data from NCBI gene datasets and its application to an update of human gene statistics. Database (Oxford) *2016*, baw153.

2. Finan, C., Gaulton, A., Kruger, F.A., Lumbers, R.T., Shah, T., Engmann, J., Galver, L., Kelley, R., Karlsson, A., Santos, R., et al. (2017). The druggable genome and support for target identification and validation in drug development. Sci. Transl. Med. *9*, eaag1166.

3. Kim, J., Hu, C., Moufawad El Achkar, C., Black, L.E., Douville, J., Larson, A., Pendergast, M.K., Goldkind, S.F., Lee, E.A., Kuniholm, A., et al. (2019). Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease. N. Engl. J. Med. *381*, 1644–1652.
4. Brenner, S., Jacob, F., and Meselson, M. (1961). An Unstable Intermediate Carrying Information from Genes to Ribosomes for Protein Synthesis. Nature *190*, 576–581.
5. Gros, F., Hiatt, H., Gilbert, W., Kurland, C.G., Risebrough, R.W., and Watson, J.D. (1961). Unstable Ribonucleic Acid Revealed by Pulse Labelling of Escherichia Coli. Nature *190*, 581–585.
6. Dias, N., Dheur, S., Nielsen, P.E., Gryaznov, S., Van Aerschot, A., Herdewijn, P., Hélène, C., and Saison-Behmoaras, T.E. (1999). Antisense PNA tridecamers targeted to the coding region of Ha-ras mRNA arrest polypeptide chain elongation. J. Mol. Biol. *294*, 403–416.
7. Cerritelli, S.M., and Crouch, R.J. (2009). Ribonuclease H: the enzymes in eukaryotes. FEBS J. *276*, 1494–1505.
8. Liang, X.H., Sun, H., Nichols, J.G., and Crooke, S.T. (2017). RNase H1-Dependent Antisense Oligonucleotides Are Robustly Active in Directing RNA Cleavage in Both the Cytoplasm and the Nucleus. Mol. Ther. *25*, 2075–2092.
9. Lennox, K.A., and Behlke, M.A. (2016). Cellular localization of long non-coding RNAs affects silencing by RNAi more than by antisense oligonucleotides. Nucleic Acids Res. *44*, 863–877.
10. Rinaldi, C., and Wood, M.J.A. (2018). Antisense oligonucleotides: the next frontier for treatment of neurological disorders. Nat. Rev. Neurol. *14*, 9–21.
11. Damase, T.R., Sukhovershin, R., Boada, C., Taraballi, F., Pettigrew, R.I., and Cooke, J.P. (2021). The Limitless Future of RNA Therapeutics. Front. Bioeng. Biotechnol. *9*, 628137.
12. Liang, X.H., Sun, H., Shen, W., Wang, S., Yao, J., Migawa, M.T., Bui, H.H., Damle, S.S., Riney, S., Graham, M.J., et al. (2017). Antisense oligonucleotides targeting translation inhibitory elements in 5' UTRs can selectively increase protein levels. Nucleic Acids Res. *45*, 9528–9546.
13. Stephenson, M.L., and Zamecnik, P.C. (1978). Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc. Natl. Acad. Sci. USA *75*, 285–288.
14. Donis-Keller, H. (1979). Site specific enzymatic cleavage of RNA. Nucleic Acids Res. *7*, 179–192.
15. Opalinska, J.B., and Gewirtz, A.M. (2002). Nucleic-acid therapeutics: basic principles and recent applications. Nat. Rev. Drug Discov. *1*, 503–514.
16. Eckstein, F. (1966). Nucleoside Phosphorothioates. J. Am. Chem. Soc. *88*, 4292–4294.
17. Martin, P. (1995). Ein neuer Zugang zu 2′-O-Alkylribonucleosiden und Eigenschaften deren Oligonucleotide. Helv. Chim. Acta *78*, 486–504.
18. Nielsen, P.E., Egholm, M., Berg, R.H., and Buchardt, O. (1991). Sequence-Selective Recognition of DNA by Strand Displacement with a Thymine-Substituted Polyamide. Science *254*, 1497–1500.
19. Hudziak, R.M., Barofsky, E., Barofsky, D.F., Weller, D.L., Huang, S.B., and Weller, D.D. (1996). Resistance of morpholino phosphorodiamidate oligomers to enzymatic degradation. Antisense Nucleic Acid Drug Dev. *6*, 267–272.
20. Obika, S., Nanbu, D., Hari, Y., Andoh, J.-I., Morio, K.-I., Doi, T., and Imanishi, T. (1998). Stability and structural features of the duplexes containing nucleoside analogues with a fixed N-type conformation, 2′-O,4′-C-methyleneribonucleosides. Tetrahedron Lett. *39*, 5401–5404.
21. Yin, H., Moulton, H.M., Seow, Y., Boyd, C., Boutilier, J., Iverson, P., and Wood, M.J.A. (2008). Cell-penetrating peptide-conjugated antisense oligonucleotides restore systemic muscle and cardiac dystrophin expression and function. Hum. Mol. Genet. *17*, 3909–3918.
22. Song, E., Zhu, P., Lee, S.K., Chowdhury, D., Kussman, S., Dykxhoorn, D.M., Feng, Y., Palliser, D., Weiner, D.B., Shankar, P., et al. (2005). Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. Nat. Biotechnol. *23*, 709–717.
23. Tanaka, K., Okuda, T., Kasahara, Y., and Obika, S. (2021). Base-modified aptamers obtained by cell-internalization SELEX facilitate cellular uptake of an antisense oligonucleotide. Mol. Ther. Nucleic Acids *23*, 440–449.
24. Hu, B., Zhong, L., Weng, Y., Peng, L., Huang, Y., Zhao, Y., and Liang, X.-J. (2020). Therapeutic siRNA: state of the art. Signal Transduct. Targeted Ther. *5*, 101.
25. Wittrup, A., and Lieberman, J. (2015). Knocking down disease: a progress report on siRNA therapeutics. Nat. Rev. Genet. *16*, 543–552.
26. Dana, H., Chalbatani, G.M., Mahmoodzadeh, H., Karimloo, R., Rezaiean, O., Moradzadeh, A., Mehmandoost, N., Moazzen, F., Mazraeh, A., Marmari, V., et al. (2017). Molecular Mechanisms and Biological Functions of siRNA. Int. J. Biomed. Sci. *13*, 48–57.
27. Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature *411*, 494–498.
28. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*, 806–811.
29. Paunovska, K., Loughrey, D., and Dahlman, J.E. (2022). Drug delivery systems for RNA therapeutics. Nat. Rev. Genet. *23*, 265–280.
30. Song, E., Zhu, P., Lee, S.-K., Chowdhury, D., Kussman, S., Dykxhoorn, D.M., Feng, Y., Palliser, D., Weiner, D.B., Shankar, P., et al. (2005). Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. Nat. Biotechnol. *23*, 709–717.
31. Ahn, I., Kang, C.S., and Han, J. (2023). Where should siRNAs go: applicable organs for siRNA drugs. Exp. Mol. Med. *55*, 1283–1292.
32. Springer, A.D., and Dowdy, S.F. (2018). GalNAc-siRNA Conjugates: Leading the Way for Delivery of RNAi Therapeutics. Nucleic Acid Therapeut. *28*, 109–118.
33. Geary, R.S., Henry, S.P., and Grillone, L.R. (2002). clinical pharmacology and potential drug interactions. Clin. Pharmacokinet. *41*, 255–260.
34. Santos, R.D., Raal, F.J., Donovan, J.M., and Cromwell, W.C. (2015). Mipomersen preferentially reduces small low-density lipoprotein particle number in patients with hypercholesterolemia. J. Clin. Lipidol. *9*, 201–209.
35. Mercuri, E., Darras, B.T., Chiriboga, C.A., Day, J.W., Campbell, C., Connolly, A.M., Iannaccone, S.T., Kirschner, J., Kuntz, N.L., Saito, K., et al. (2018). Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. N. Engl. J. Med. *378*, 625–635.
36. Mendell, J.R., Rodino-Klapac, L.R., Sahenk, Z., Roush, K., Bird, L., Lowes, L.P., Alfano, L., Gomez, A.M., Lewis, S., Kota, J., et al. (2013). Eteplirsen for the treatment of Duchenne muscular dystrophy. Ann. Neurol. *74*, 637–647.
37. Benson, M.D., Waddington-Cruz, M., Berk, J.L., Polydefkis, M., Dyck, P.J., Wang, A.K., Planté-Bordeneuve, V., Barroso, F.A., Merlini, G., Obici, L., et al. (2018). Inotersen Treatment for Patients with Hereditary Transthyretin Amyloidosis. N. Engl. J. Med. *379*, 22–31.
38. Scaglioni, D., Catapano, F., Ellis, M., Torelli, S., Chambers, D., Feng, L., Beck, M., Sewry, C., Monforte, M., Harriman, S., et al. (2021). The administration of antisense oligonucleotide golodirsen reduces pathological regeneration in patients with Duchenne muscular dystrophy. Acta Neuropathol. Commun. *9*, 7.
39. Clemens, P.R., Rao, V.K., Connolly, A.M., Harper, A.D., Mah, J.K., McDonald, C.M., Smith, E.C., Zaidman, C.M., Nakagawa, T.; CINRG DNHS Investigators, and Hoffman, E.P. (2022). Long-Term Functional Efficacy and Safety of Viltolarsen in Patients with Duchenne Muscular Dystrophy. J. Neuromuscul. Dis. *9*, 493–501.
40. Shirley, M. (2021). Casimersen: First Approval. Drugs *81*, 875–879.
41. Coelho, T., Marques, W., Jr., Dasgupta, N.R., Chao, C.C., Parman, Y., França, M.C., Jr., Guo, Y.C., Wixner, J., Ro, L.S., Calandra, C.R., et al. (2023). Eplontersen for Hereditary Transthyretin Amyloidosis With Polyneuropathy. JAMA *330*, 1448–1458.
42. Kristen, A.V., Ajroud-Driss, S., Conceição, I., Gorevic, P., Kyriakides, T., and Obici, L.P. (2019). an RNAi therapeutic for the treatment of hereditary transthyretin-mediated amyloidosis. Neurodegener. Dis. Manag. *9*, 5–23.
43. Balwani, M., Sardh, E., Ventura, P., Peiró, P.A., Rees, D.C., Stölzel, U., Bissell, D.M., Bonkovsky, H.L., Windyga, J., Anderson, K.E., et al. (2020). Phase 3 Trial of RNAi Therapeutic Givosiran for Acute Intermittent Porphyria. N. Engl. J. Med. *382*, 2289–2301.

44. Liebow, A., Li, X., Racie, T., Hettinger, J., Bettencourt, B.R., Najafian, N., Haslett, P., Fitzgerald, K., Holmes, R.P., Erbe, D., et al. (2017). An Investigational RNAi Therapeutic Targeting Glycolate Oxidase Reduces Oxalate Production in Models of Primary Hyperoxaluria. J. Am. Soc. Nephrol. *28*, 494–503.

45. Lamb, Y.N. (2021). Inclisiran: First Approval. Drugs *81*, 389–395.

46. Goldfarb, D.S., Lieske, J.C., Groothoff, J., Schalk, G., Russell, K., Yu, S., and Vrhnjak, B. (2023). Nedosiran in primary hyperoxaluria subtype 3: results from a phase I, single-dose study (PHYOX4). Urolithiasis *51*, 80.

47. Gragoudas, E.S., Adamis, A.P., Cunningham, E.T., Jr., Feinsod, M., and Guyer, D.R.; VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group (2004). Pegaptanib for neovascular age-related macular degeneration. N. Engl. J. Med. *351*, 2805–2816.

48. Jaffe, G.J., Westby, K., Csaky, K.G., Monés, J., Pearlman, J.A., Patel, S.S., Joondeph, B.C., Randolph, J., Masonson, H., and Rezaei, K.A. (2021). C5 Inhibitor Avacincaptad Pegol for Geographic Atrophy Due to Age-Related Macular Degeneration: A Randomized Pivotal Phase 2/3 Trial. Ophthalmology *128*, 576–586.

49. Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Pérez Marc, G., Moreira, E.D., Zerbini, C., et al. (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N. Engl. J. Med. *383*, 2603–2615.

50. Baden, L.R., El Sahly, H.M., Essink, B., Kotloff, K., Frey, S., Novak, R., Diemert, D., Spector, S.A., Rouphael, N., Creech, C.B., et al. (2021). Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N. Engl. J. Med. Overseas. Ed. *384*, 403–416.

51. Frangoul, H., Locatelli, F., Sharma, A., Bhatia, M., Mapara, M., Molinari, L., Wall, D., Liem, R.I., Telfer, P., Shah, A.J., et al. (2024). Exagamglogene Autotemcel for Severe Sickle Cell Disease. N. Engl. J. Med. *390*, 1649–1662.

52. Scott, L.J. (2020). Givosiran: First Approval. Drugs *80*, 335–339.

53. Huang, Y., Zheng, S., Guo, Z., de Mollerat du Jeu, X., Liang, X.-J., Yang, Z., Zhang, H.-Y., Gao, S., and Liang, Z. (2022). Ionizable liposomal siRNA therapeutics enables potent and persistent treatment of Hepatitis B. Signal Transduct. Targeted Ther. *7*, 38.

54. Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Acsadi, G., Jani, A., and Felgner, P.L. (1990). Direct Gene Transfer into Mouse Muscle in Vivo. Science *247*, 1465–1468.

55. Jirikowski, G.F., Sanna, P.P., Maciejewski-Lenoir, D., and Bloom, F.E. (1992). Reversal of diabetes insipidus in Brattleboro rats: intrahypothalamic injection of vasopressin mRNA. Science *255*, 996–998.

56. Karikó, K., Muramatsu, H., Welsh, F.A., Ludwig, J., Kato, H., Akira, S., and Weissman, D. (2008). Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol. Ther. *16*, 1833–1840.

57. Leppek, K., Byeon, G.W., Kladwang, W., Wayment-Steele, H.K., Kerr, C.H., Xu, A.F., Kim, D.S., Topkar, V.V., Choe, C., Rothschild, D., et al. (2022). Combinatorial optimization of mRNA structure, stability, and translation for RNA-based therapeutics. Nat. Commun. *13*, 1536.

58. Dubin, D.T., and Stollar, V. (1975). Methylation of sindbis virus "26S" messenger RNA. Biochem. Biophys. Res. Commun. *66*, 1373–1379.

59. Zhang, Y., Zhang, L.-S., Dai, Q., Chen, P., Lu, M., Kairis, E.L., Murugaiah, V., Xu, J., Shukla, R.K., Liang, X., et al. (2022). 5-methylcytosine (m^5C) RNA modification controls the innate immune response to virus infection by regulating type I interferons. Proc. Natl. Acad. Sci. USA *119*, e2123338119.

60. Nance, K.D., and Meier, J.L. (2021). Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. ACS Cent. Sci. *7*, 748–756.

61. Boo, S.H., and Kim, Y.K. (2020). The emerging role of RNA modifications in the regulation of mRNA stability. Exp. Mol. Med. *52*, 400–408.

62. Guo, G., Pan, K., Fang, S., Ye, L., Tong, X., Wang, Z., Xue, X., and Zhang, H. (2021). Advances in mRNA 5-methylcytosine modifications: Detection, effectors, biological functions, and clinical relevance. Mol. Ther. Nucleic Acids *26*, 575–593.

63. Weng, Y., Li, C., Yang, T., Hu, B., Zhang, M., Guo, S., Xiao, H., Liang, X.J., and Huang, Y. (2020). The challenge and prospect of mRNA therapeutics landscape. Biotechnol. Adv. *40*, 107534.

64. Ripoll, M., Bernard, M.-C., Vaure, C., Bazin, E., Commandeur, S., Perkov, V., Lemdani, K., Nicolaï, M.-C., Bonifassi, P., Kichler, A., et al. (2022). An imidazole modified lipid confers enhanced mRNA-LNP stability and strong immunization properties in mice and non-human primates. Biomaterials *286*, 121570.

65. Travieso, T., Li, J., Mahesh, S., Mello, J.D.F.R.E., and Blasi, M. (2022). The use of viral vectors in vaccine development. npj Vaccines *7*, 75.

66. Liang, D., and Wilusz, J.E. (2014). Short intronic repeat sequences facilitate circular RNA production. Genes Dev. *28*, 2233–2247.

67. Tang, T.H., Rozhdestvensky, T.S., d'Orval, B.C., Bortolin, M.L., Huber, H., Charpentier, B., Branlant, C., Bachellerie, J.P., Brosius, J., and Hüttenhofer, A. (2002). RNomics in Archaea reveals a further link between splicing of archaeal introns and rRNA processing. Nucleic Acids Res. *30*, 921–930.

68. Jeck, W.R., Sorrentino, J.A., Wang, K., Slevin, M.K., Burd, C.E., Liu, J., Marzluff, W.F., and Sharpless, N.E. (2013). Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA *19*, 141–157.

69. Chen, L.L., and Yang, L. (2015). Regulation of circRNA biogenesis. RNA Biol. *12*, 381–388.

70. Hansen, T.B., Jensen, T.I., Clausen, B.H., Bramsen, J.B., Finsen, B., Damgaard, C.K., and Kjems, J. (2013). Natural RNA circles function as efficient microRNA sponges. Nature *495*, 384–388.

71. Yao, D., Lin, S., Chen, S., and Wang, Z. (2022). circHIPK3 regulates cell proliferation and migration by sponging microRNA-124 and regulating serine/threonine kinase 3 expression in esophageal squamous cell carcinoma. Bioengineered *13*, 9767–9780.

72. Zang, J., Lu, D., and Xu, A. (2020). The interaction of circRNAs and RNA binding proteins: An important part of circRNA maintenance and function. J. Neurosci. Res. *98*, 87–97.

73. Wang, Y., and Wang, Z. (2015). Efficient backsplicing produces translatable circular mRNAs. RNA *21*, 172–179.

74. Wesselhoeft, R.A., Kowalski, P.S., Parker-Hale, F.C., Huang, Y., Bisaria, N., and Anderson, D.G. (2019). RNA Circularization Diminishes Immunogenicity and Can Extend Translation Duration In Vivo. Mol. Cell *74*, 508–520.e4.

75. Breuer, J., Barth, P., Noe, Y., Shalamova, L., Goesmann, A., Weber, F., and Rossbach, O. (2022). What goes around comes around: Artificial circular RNAs bypass cellular antiviral responses. Mol. Ther. Nucleic Acids *28*, 623–635.

76. He, A.T., Liu, J., Li, F., and Yang, B.B. (2021). Targeting circular RNAs as a therapeutic approach: current strategies and challenges. Signal Transduct. Targeted Ther. *6*, 185.

77. Wang, Z., Ma, K., Cheng, Y., Abraham, J.M., Liu, X., Ke, X., Wang, Z., and Meltzer, S.J. (2019). Synthetic circular multi-miR sponge simultaneously inhibits miR-21 and miR-93 in esophageal carcinoma. Lab. Invest. *99*, 1442–1453.

78. Liu, X., Abraham, J.M., Cheng, Y., Wang, Z., Wang, Z., Zhang, G., Ashktorab, H., Smoot, D.T., Cole, R.N., Boronina, T.N., et al. (2018). Synthetic Circular RNA Functions as a miR-21 Sponge to Suppress Gastric Carcinoma Cell Proliferation. Mol. Ther. Nucleic Acids *13*, 312–321.

79. Chen, R., Wang, S.K., Belk, J.A., Amaya, L., Li, Z., Cardenas, A., Abe, B.T., Chen, C.-K., Wender, P.A., and Chang, H.Y. (2023). Engineering circular RNA for enhanced protein production. Nat. Biotechnol. *41*, 262–272.

80. Wu, D.P., Zhao, Y.D., Yan, Q.Q., Liu, L.L., Wei, Y.S., and Huang, J.L. (2023). Circular RNAs: emerging players in brain aging and neurodegenerative diseases. J. Pathol. *259*, 1–9.

81. Wang, W., Sun, L., Huang, M.T., Quan, Y., Jiang, T., Miao, Z., and Zhang, Q. (2023). Regulatory circular RNAs in viral diseases: applications in diagnosis and therapy. RNA Biol. *20*, 847–858.

82. Schult, P., Roth, H., Adams, R.L., Mas, C., Imbert, L., Orlik, C., Ruggieri, A., Pyle, A.M., and Lohmann, V. (2018). microRNA-122 amplifies hepatitis C virus translation by shaping the structure of the internal ribosomal entry site. Nat. Commun. *9*, 2613.

83. Zhou, Z., Zhang, R., Li, X., Zhang, W., Zhan, Y., Lang, Z., Tao, Q., Yu, J., Yu, S., Yu, Z., and Zheng, J. (2024). Circular RNA cVIM promotes hepatic stellate cell activation in liver fibrosis via miR-122-5p/miR-9-5p-mediated TGF-β signaling cascade. Commun. Biol. *7*, 113.

84. Amaya, L., Grigoryan, L., Li, Z., Lee, A., Wender, P.A., Pulendran, B., and Chang, H.Y. (2023). Circular RNA vaccine induces potent T cell responses. Proc. Natl. Acad. Sci. USA *120*, e2302191120.

85. O'Brien, J., Hayder, H., Zayed, Y., and Peng, C. (2018). Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front. Endocrinol. *9*, 402.

86. Lam, J.K.W., Chow, M.Y.T., Zhang, Y., and Leung, S.W.S. (2015). siRNA Versus miRNA as Therapeutics for Gene Silencing. Mol. Ther. Nucleic Acids *4*, e252.

87. Hermeking, H. (2010). The miR-34 family in cancer and apoptosis. Cell Death Differ. *17*, 193–199.

88. Zhang, J.G., Wang, J.J., Zhao, F., Liu, Q., Jiang, K., and Yang, G.H. (2010). MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin. Chim. Acta *411*, 846–852.

89. Hong, D.S., Kang, Y.-K., Borad, M., Sachdev, J., Ejadi, S., Lim, H.Y., Brenner, A.J., Park, K., Lee, J.-L., Kim, T.-Y., et al. (2020). Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. Br. J. Cancer *122*, 1630–1637.

90. Huang, Y. (2017). Preclinical and Clinical Advances of GalNAc-Decorated Nucleic Acid Therapeutics. Mol. Ther. Nucleic Acids *6*, 116–132.

91. Ellington, A.D., and Szostak, J.W. (1990). In vitro selection of RNA molecules that bind specific ligands. Nature *346*, 818–822.

92. Tuerk, C., and Gold, L. (1990). Systematic Evolution of Ligands by Exponential Enrichment: RNA Ligands to Bacteriophage T4 DNA Polymerase. Science *249*, 505–510.

93. Zhou, J., and Rossi, J. (2017). Aptamers as targeted therapeutics: current potential and challenges. Nat. Rev. Drug Discov. *16*, 181–202.

94. Mi, J., Liu, Y., Rabbani, Z.N., Yang, Z., Urban, J.H., Sullenger, B.A., and Clary, B.M. (2010). In vivo selection of tumor-targeting RNA motifs. Nat. Chem. Biol. *6*, 22–24.

95. Yunn, N.-O., Lee, J., Lee, H.S., Oh, E.J., Park, M., Park, S., Jin, S.Y., Shin, E., Lee, J.w.y., Kim, Y., et al. (2022). An aptamer agonist of the insulin receptor acts as a positive or negative allosteric modulator, depending on its concentration. Exp. Mol. Med. *54*, 531–541.

96. Tan, J., Yang, N., Zhong, L., Tan, J., Hu, Z., Zhao, Q., Gong, W., Zhang, Z., Zheng, R., Lai, Z., et al. (2017). A New Theranostic System Based on Endoglin Aptamer Conjugated Fluorescent Silica Nanoparticles. Theranostics *7*, 4862–4876.

97. Spöring, M., Boneberg, R., and Hartig, J.S. (2020). Aptamer-Mediated Control of Polyadenylation for Gene Expression Regulation in Mammalian Cells. ACS Synth. Biol. *9*, 3008–3018.

98. Spöring, M., Finke, M., and Hartig, J.S. (2020). Aptamers in RNA-based switches of gene expression. Curr. Opin. Biotechnol. *63*, 34–40.

99. Vogel, M., Weigand, J.E., Kluge, B., Grez, M., and Suess, B. (2018). portable RNA device for the control of exon skipping in mammalian cells. Nucleic Acids Res. *46*, e48.

100. Suess, B., Hanson, S., Berens, C., Fink, B., Schroeder, R., and Hillen, W. (2003). Conditional gene expression by controlling translation with tetracycline-binding aptamers. Nucleic Acids Res. *31*, 1853–1858.

101. Strobel, B., Düchs, M.J., Blazevic, D., Rechtsteiner, P., Braun, C., Baum-Kroker, K.S., Schmid, B., Ciossek, T., Gottschling, D., Hartig, J.S., and Kreuz, S. (2020). A Small-Molecule-Responsive Riboswitch Enables Conditional Induction of Viral Vector-Mediated Gene Expression in Mice. ACS Synth. Biol. *9*, 1292–1305.

102. Healy, J.M., Lewis, S.D., Kurz, M., Boomer, R.M., Thompson, K.M., Wilson, C., and McCauley, T.G. (2004). Pharmacokinetics and biodistribution of novel aptamer compositions. Pharm. Res. (N. Y.) *21*, 2234–2246.

103. Musumeci, D., and Montesarchio, D. (2012). Polyvalent nucleic acid aptamers and modulation of their activity: a focus on the thrombin binding aptamer. Pharmacol. Ther. *136*, 202–215.

104. Guo, P. (2010). The emerging field of RNA nanotechnology. Nat. Nanotechnol. *5*, 833–842.

105. Dass, C.R., Saravolac, E.G., Li, Y., and Sun, L.Q. (2002). Cellular uptake, distribution, and stability of 10-23 deoxyribozymes. Antisense Nucleic Acid Drug Dev. *12*, 289–299.

106. Boomer, R.M., Lewis, S.D., Healy, J.M., Kurz, M., Wilson, C., and McCauley, T.G. (2005). Conjugation to polyethylene glycol polymer promotes aptamer biodistribution to healthy and inflamed tissues. Oligonucleotides *15*, 183–195.

107. Willis, M.C., Collins, B.D., Zhang, T., Green, L.S., Sebesta, D.P., Bell, C., Kellogg, E., Gill, S.C., Magallanez, A., Knauer, S., et al. (1998). Liposome-anchored vascular endothelial growth factor aptamers. Bioconjugate Chem. *9*, 573–582.

108. Burmeister, P.E., Lewis, S.D., Silva, R.F., Preiss, J.R., Horwitz, L.R., Pendergrast, P.S., McCauley, T.G., Kurz, J.C., Epstein, D.M., Wilson, C., and Keefe, A.D. (2005). Direct *in vitro* selection of a 2'-O-methyl aptamer to VEGF. Chem. Biol. *12*, 25–33.

109. Lee, C.H., Lee, S.-H., Kim, J.H., Noh, Y.-H., Noh, G.-J., and Lee, S.-W. (2015). Pharmacokinetics of a Cholesterol-conjugated Aptamer Against the Hepatitis C Virus (HCV) NS5B Protein. Mol. Ther. Nucleic Acids *4*, e254.

110. Zhou, J., Soontornworajit, B., Martin, J., Sullenger, B.A., Gilboa, E., and Wang, Y. (2009). A Hybrid DNA Aptamer–Dendrimer Nanomaterial for Targeted Cell Labeling. Macromol. Biosci. *9*, 831–835.

111. Jo, H., and Ban, C. (2016). Aptamer–nanoparticle complexes as powerful diagnostic and therapeutic tools. Exp. Mol. Med. *48*, e230.

112. Bauer, M., Strom, M., Hammond, D.S., and Shigdar, S. (2019). Anything You Can Do, I Can Do Better: Can Aptamers Replace Antibodies in Clinical Diagnostic Applications? Molecules *24*, 4377.

113. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell *35*, 849–857.

114. Zaug, A.J., and Cech, T.R. (1986). The intervening sequence RNA of Tetrahymena is an enzyme. Science *231*, 470–475.

115. Forster, A.C., and Symons, R.H. (1987). Self-cleavage of plus and minus RNAs of a virusoid and a structural model for the active sites. Cell *49*, 211–220.

116. Hampel, A., Tritz, R., Hicks, M., and Cruz, P. (1990). Hairpin' catalytic RNA model: evidence for helices and sequence requirement for substrate RNA. Nucleic Acids Res. *18*, 299–304.

117. Kashani-Sabet, M. (2002). Ribozyme therapeutics. J. Invest. Dermatol. Symp. Proc. *7*, 76–78.

118. Sarver, N., Cantin, E.M., Chang, P.S., Zaia, J.A., Ladne, P.A., Stephens, D.A., and Rossi, J.J. (1990). Ribozymes as potential anti-HIV-1 therapeutic agents. Science *247*, 1222–1225.

119. Scanlon, K.J., Jiao, L., Funato, T., Wang, W., Tone, T., Rossi, J.J., and Kashani-Sabet, M. (1991). Ribozyme-mediated cleavage of c-fos mRNA reduces gene expression of DNA synthesis enzymes and metallothionein. Proc. Natl. Acad. Sci. USA *88*, 10591–10595.

120. Sullivan, S.M. (1994). Development of ribozymes for gene therapy. J. Invest. Dermatol. *103*, 85s–89s.

121. Weng, D.E., Masci, P.A., Radka, S.F., Jackson, T.E., Weiss, P.A., Ganapathi, R., Elson, P.J., Capra, W.B., Parker, V.P., Lockridge, J.A., et al. (2005). A phase I clinical trial of a ribozyme-based angiogenesis inhibitor targeting vascular endothelial growth factor receptor-1 for patients with refractory solid tumors. Mol. Cancer Therapeut. *4*, 948–955.

122. Morrow, P.K., Murthy, R.K., Ensor, J.D., Gordon, G.S., Margolin, K.A., Elias, A.D., Urba, W.J., Weng, D.E., Rugo, H.S., and Hortobagyi, G.N. (2012). An open-label, phase 2 trial of RPI.4610 (Angiozyme) in the treatment of metastatic breast cancer. Cancer *118*, 4098–4104.

123. Heidenreich, O., Benseler, F., Fahrenholz, A., and Eckstein, F. (1994). High activity and stability of hammerhead ribozymes containing 2'-modified pyrimidine nucleosides and phosphorothioates. J. Biol. Chem. *269*, 2131–2138.

124. Czapik, T., Piasecka, J., Kierzek, R., and Kierzek, E. (2022). Structural variants and modifications of hammerhead ribozymes targeting influenza A virus conserved structural motifs. Mol. Ther. Nucleic Acids *29*, 64–74.

125. Peng, Z., Cheng, Y., Tan, B.C.-M., Kang, L., Tian, Z., Zhu, Y., Zhang, W., Liang, Y., Hu, X., Tan, X., et al. (2012). Comprehensive analysis of RNA-Seq data reveals extensive RNA editing in a human transcriptome. Nat. Biotechnol. *30*, 253–260.

126. Vu, L.T., and Tsukahara, T. (2017). C-to-U editing and site-directed RNA editing for the correction of genetic mutations. Biosci. Trends *11*, 243–253.

127. Rajappa-Titu, L., Suematsu, T., Munoz-Tello, P., Long, M., Demir, Ö., Cheng, K.J., Stagno, J.R., Luecke, H., Amaro, R.E., Aphasizheva, I., et al. (2016). RNA Editing TUTase 1: structural foundation of substrate recognition, complex interactions and drug targeting. Nucleic Acids Res. *44*, 10862–10878.

128. Nishikura, K. (2016). A-to-I editing of coding and non-coding RNAs by ADARs. Nat. Rev. Mol. Cell Biol. *17*, 83–96.

129. Blanc, V., and Davidson, N.O. (2003). C-to-U RNA editing: mechanisms leading to genetic diversity. J. Biol. Chem. *278*, 1395–1398.

130. Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Joung, J., and Zhang, F. (2017). RNA editing with CRISPR-Cas13. Science *358*, 1019–1027.

131. Fukuda, M., Umeno, H., Nose, K., Nishitarumizu, A., Noguchi, R., and Nakagawa, H. (2017). Construction of a guide-RNA for site-directed RNA mutagenesis utilising intracellular A-to-I RNA editing. Sci. Rep. *7*, 41478.

132. Heep, M., Mach, P., Reautschnig, P., Wettengel, J., and Stafforst, T. (2017). Applying Human ADAR1p110 and ADAR1p150 for Site-Directed RNA Editing-G/C Substitution Stabilizes GuideRNAs against Editing. Genes *8*, 34.

133. Montiel-Gonzalez, M.F., Vallecillo-Viejo, I., Yudowski, G.A., and Rosenthal, J.J.C. (2013). Correction of mutations within the cystic fibrosis transmembrane conductance regulator by site-directed RNA editing. Proc. Natl. Acad. Sci. USA *110*, 18285–18290.

134. Hanswillemenke, A., Kuzdere, T., Vogel, P., Jékely, G., and Stafforst, T. (2015). Site-Directed RNA Editing in Vivo Can Be Triggered by the Light-Driven Assembly of an Artificial Riboprotein. J. Am. Chem. Soc. *137*, 15875–15881.

135. Zhou, C., Sun, Y., Yan, R., Liu, Y., Zuo, E., Gu, C., Han, L., Wei, Y., Hu, X., Zeng, R., et al. (2019). Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. Nature *571*, 275–278.

136. Charlesworth, C.T., Deshpande, P.S., Dever, D.P., Camarena, J., Lemgart, V.T., Cromer, M.K., Vakulskas, C.A., Collingwood, M.A., Zhang, L., Bode, N.M., et al. (2019). Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat. Med. *25*, 249–254.

137. Chew, W.L., Tabebordbar, M., Cheng, J.K.W., Mali, P., Wu, E.Y., Ng, A.H.M., Zhu, K., Wagers, A.J., and Church, G.M. (2016). A multifunctional AAV–CRISPR–Cas9 and its host response. Nat. Methods *13*, 868–874.

138. Merkle, T., Merz, S., Reautschnig, P., Blaha, A., Li, Q., Vogel, P., Wettengel, J., Li, J.B., and Stafforst, T. (2019). Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides. Nat. Biotechnol. *37*, 133–138.

139. Qu, L., Yi, Z., Zhu, S., Wang, C., Cao, Z., Zhou, Z., Yuan, P., Yu, Y., Tian, F., Liu, Z., et al. (2019). Programmable RNA editing by recruiting endogenous ADAR using engineered RNAs. Nat. Biotechnol. *37*, 1059–1069.

140. Yi, Z., Qu, L., Tang, H., Liu, Z., Liu, Y., Tian, F., Wang, C., Zhang, X., Feng, Z., Yu, Y., et al. (2022). Engineered circular ADAR-recruiting RNAs increase the efficiency and fidelity of RNA editing *in vitro* and *in vivo*. Nat. Biotechnol. *40*, 946–955.

141. Wong, S.K., Sato, S., and Lazinski, D.W. (2001). Substrate recognition by ADAR1 and ADAR2. RNA *7*, 846–858.

142. Tian, N., Yang, Y., Sachsenmaier, N., Muggenhumer, D., Bi, J., Waldsich, C., Jantsch, M.F., and Jin, Y. (2011). A structural determinant required for RNA editing. Nucleic Acids Res. *39*, 5669–5681.

143. Ensterö, M., Daniel, C., Wahlstedt, H., Major, F., and Ohman, M. (2009). Recognition and coupling of A-to-I edited sites are determined by the tertiary structure of the RNA. Nucleic Acids Res. *37*, 6916–6926.

144. Reautschnig, P., Wahn, N., Wettengel, J., Schulz, A.E., Latifi, N., Vogel, P., Kang, T.W., Pfeiffer, L.S., Zarges, C., Naumann, U., et al. (2022). CLUSTER guide RNAs enable precise and efficient RNA editing with endogenous ADAR enzymes *in vivo*. Nat. Biotechnol. *40*, 759–768.

145. Katrekar, D., Yen, J., Xiang, Y., Saha, A., Meluzzi, D., Savva, Y., and Mali, P. (2022). Efficient *in vitro* and *in vivo* RNA editing via recruitment of endogenous ADARs using circular guide RNAs. Nat. Biotechnol. *40*, 938–945.

146. Monian, P., Shivalila, C., Lu, G., Shimizu, M., Boulay, D., Bussow, K., Byrne, M., Bezigian, A., Chatterjee, A., Chew, D., et al. (2022). Endogenous ADAR-mediated RNA editing in non-human primates using stereopure chemically modified oligonucleotides. Nat. Biotechnol. *40*, 1093–1102.

147. Smargon, A.A., Cox, D.B.T., Pyzocha, N.K., Zheng, K., Slaymaker, I.M., Gootenberg, J.S., Abudayyeh, O.A., Essletzbichler, P., Shmakov, S., Makarova, K.S., et al. (2017). Cas13b Is a Type VI-B CRISPR-Associated RNA-Guided RNase Differentially Regulated by Accessory Proteins Csx27 and Csx28. Mol. Cell *65*, 618–630.e7.

148. Shmakov, S., Abudayyeh, O.O., Makarova, K.S., Wolf, Y.I., Gootenberg, J.S., Semenova, E., Minakhin, L., Joung, J., Konermann, S., Severinov, K., et al. (2015). Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems. Mol. Cell *60*, 385–397.

149. Perčulija, V., Lin, J., Zhang, B., and Ouyang, S. (2021). Functional Features and Current Applications of the RNA-Targeting Type VI CRISPR-Cas Systems. Adv. Sci. *8*, 2004685.

150. Dunn, M.R., Jimenez, R.M., and Chaput, J.C. (2017). Analysis of aptamer discovery and technology. Nat. Rev. Chem *1*, 0076.

151. Irvine, D., Tuerk, C., and Gold, L. (1991). Systematic evolution of ligands by exponential enrichment with integrated optimization by non-linear analysis. J. Mol. Biol. *222*, 739–761.

152. Wilson, D.S., and Szostak, J.W. (1999). In vitro selection of functional nucleic acids. Annu. Rev. Biochem. *68*, 611–647.

153. Yan, J., Xiong, H., Cai, S., Wen, N., He, Q., Liu, Y., Peng, D., and Liu, Z. (2019). Advances in aptamer screening technologies. Talanta *200*, 124–144.

154. Narayan, C., Kwon, J., Kim, C., Kim, S.J., and Jang, S.K. (2020). Virus-based SELEX (viro-SELEX) allows development of aptamers targeting knotty proteins. Analyst *145*, 1473–1482.

155. Avci-Adali, M., Metzger, M., Perle, N., Ziemer, G., and Wendel, H.P. (2010). Pitfalls of cell-systematic evolution of ligands by exponential enrichment (SELEX): existing dead cells during *in vitro* selection anticipate the enrichment of specific aptamers. Oligonucleotides *20*, 317–323.

156. Bakhtiari, H., Palizban, A.A., Khanahmad, H., and Mofid, M.R. (2021). Novel Approach to Overcome Defects of Cell-SELEX in Developing Aptamers against Aspartate β-Hydroxylase. ACS Omega *6*, 11005–11014.

157. Zhong, Y., Zhao, J., Li, J., Liao, X., and Chen, F. (2020). Advances of aptamers screened by Cell-SELEX in selection procedure, cancer diagnostics and therapeutics. Anal. Biochem. *598*, 113620.

158. Raddatz, M.S.L., Dolf, A., Endl, E., Knolle, P., Famulok, M., and Mayer, G. (2008). Enrichment of cell-targeting and population-specific aptamers by fluorescence-activated cell sorting. Angew. Chem. *47*, 5190–5193.

159. Zhang, Y., Lai, B.S., and Juhas, M. (2019). Recent Advances in Aptamer Discovery and Applications. Molecules *24*, 941.

160. Zhang, J., Zhu, A., Mei, M., Qu, J., Huang, Y., Shi, Y., Xue, M., Zhang, J., Zhang, R., Zhou, B., et al. (2023). Repurposing CRISPR/Cas to Discover SARS-CoV-2 Detecting and Neutralizing Aptamers. Adv Sci (Weinh) *10*, 2300656.

161. Eckstein, F. (2000). Phosphorothioate oligodeoxynucleotides: what is their origin and what is unique about them? Antisense Nucleic Acid Drug Dev. *10*, 117–121.

162. Geary, R.S., Yu, R.Z., and Levin, A.A. (2001). Pharmacokinetics of phosphorothioate antisense oligodeoxynucleotides. Curr. Opin. Invest. Drugs *2*, 562–573.

163. Crooke, S.T. (1999). Molecular mechanisms of action of antisense drugs. Biochim. Biophys. Acta *1489*, 31–44.

164. Stein, C.A., Subasinghe, C., Shinozuka, K., and Cohen, J.S. (1988). Physicochemical properties of phosphorothioate oligodeoxynucleotides. Nucleic Acids Res. *16*, 3209–3221.

165. Verma, S., and Eckstein, F. (1998). MODIFIED OLIGONUCLEOTIDES: Synthesis and Strategy for Users. Annu. Rev. Biochem. *67*, 99–134.

166. Rifai, A., Brysch, W., Fadden, K., Clark, J., and Schlingensiepen, K.H. (1996). Clearance kinetics, biodistribution, and organ saturability of phosphorothioate oligodeoxynucleotides in mice. Am. J. Pathol. *149*, 717–725.

167. Watanabe, T.A., Geary, R.S., and Levin, A.A. (2006). Plasma protein binding of an antisense oligonucleotide targeting human ICAM-1 (ISIS 2302). Oligonucleotides *16*, 169–180.

168. Wu, H., Lima, W.F., Zhang, H., Fan, A., Sun, H., and Crooke, S.T. (2004). Determination of the role of the human RNase H1 in the pharmacology of DNA-like antisense drugs. J. Biol. Chem. *279*, 17181–17189.

169. Guvakova, M.A., Yakubov, L.A., Vlodavsky, I., Tonkinson, J.L., and Stein, C.A. (1995). Phosphorothioate oligodeoxynucleotides bind to basic fibroblast growth factor, inhibit its binding to cell surface receptors, and remove it from low affinity binding sites on extracellular matrix. J. Biol. Chem. *270*, 2620–2627.

170. Rockwell, P., O'Connor, W.J., King, K., Goldstein, N.I., Zhang, L.M., and Stein, C.A. (1997). Cell-surface perturbations of the epidermal growth factor and vascular endothelial growth factor receptors by phosphorothioate oligodeoxynucleotides. Proc. Natl. Acad. Sci. USA *94*, 6523–6528.

171. Teplova, M., Minasov, G., Tereshko, V., Inamati, G.B., Cook, P.D., Manoharan, M., and Egli, M. (1999). Crystal structure and improved antisense properties of 2'-O-(2-methoxyethyl)-RNA. Nat. Struct. Biol. *6*, 535–539.

172. Baker, B.F., Lot, S.S., Condon, T.P., Cheng-Flournoy, S., Lesnik, E.A., Sasmor, H.M., and Bennett, C.F. (1997). 2'-O-(2-Methoxy)ethyl-modified anti-intercellular adhesion molecule 1 (ICAM-1) oligonucleotides selectively increase the ICAM-1 mRNA level and inhibit formation of the ICAM-1 translation initiation complex in human umbilical vein endothelial cells. J. Biol. Chem. *272*, 11994–12000.

173. Sierakowska, H., Sambade, M.J., Agrawal, S., and Kole, R. (1996). Repair of thalassemic human β-globin mRNA in mammalian cells by antisense oligonucleotides. Proc. Natl. Acad. Sci. USA *93*, 12840–12844.

174. Koshkin, A.A., Singh, S.K., Nielsen, P., Rajwanshi, V.K., Kumar, R., Meldgaard, M., Olsen, C.E., and Wengel, J. (1998). LNA (Locked Nucleic Acids): Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition. Tetrahedron *54*, 3607–3630.

175. Obad, S., dos Santos, C.O., Petri, A., Heidenblad, M., Broom, O., Ruse, C., Fu, C., Lindow, M., Stenvang, J., Straarup, E.M., et al. (2011). Silencing of microRNA families by seed-targeting tiny LNAs. Nat. Genet. *43*, 371–378.

176. Su Young, O., YeongSoon, J., Heekyung Park Ki-Woong, C., and Yup, K. (2009). A Highly Effective and Long-Lasting Inhibition of miRNAs with PNA-Based AntisenseOligonucleotides. Mol. Cell. *28*, 341–345.

177. Kole, R., and Krieg, A.M. (2015). Exon skipping therapy for Duchenne muscular dystrophy. Adv. Drug Deliv. Rev. *87*, 104–107.

178. Summerton, J.E. (2007). Morpholino, siRNA, and S-DNA compared: impact of structure and mechanism of action on off-target effects and sequence specificity. Curr. Top. Med. Chem. *7*, 651–660.

179. Iversen, P.L. (2001). Phosphorodiamidate morpholino oligomers: favorable properties for sequence-specific gene inactivation. Curr. Opin. Mol. Therapeut. *3*, 235–238.

180. Monia, B.P., Lesnik, E.A., Gonzalez, C., Lima, W.F., McGee, D., Guinosso, C.J., Kawasaki, A.M., Cook, P.D., and Freier, S.M. (1993). Evaluation of 2'-modified oligonucleotides containing 2'-deoxy gaps as antisense inhibitors of gene expression. J. Biol. Chem. *268*, 14514–14522.

181. Aslesh, T., and Yokota, T. (2020). Development of Antisense Oligonucleotide Gapmers for the Treatment of Huntington's Disease. Methods Mol. Biol. *2176*, 57–67.

182. Gantier, M.P., and Williams, B.R.G. (2007). The response of mammalian cells to double-stranded RNA. Cytokine Growth Factor Rev. *18*, 363–371.

183. Yi, C., and Pan, T. (2011). Cellular dynamics of RNA modification. Acc. Chem. Res. *44*, 1380–1388.

184. Peacock, H., Kannan, A., Beal, P.A., and Burrows, C.J. (2011). Chemical Modification of siRNA Bases To Probe and Enhance RNA Interference. J. Org. Chem. *76*, 7295–7300.

185. Uhlmann, E., and Peyman, A. (1990). Antisense oligonucleotides: a new therapeutic principle. Chem. Rev. *90*, 543–584.

186. Micklefield, J. (2001). Backbone modification of nucleic acids: synthesis, structure and therapeutic applications. Curr. Med. Chem. *8*, 1157–1179.

187. Lennox, K.A., and Behlke, M.A. (2011). Chemical modification and design of anti-miRNA oligonucleotides. Gene Ther. *18*, 1111–1120.

188. Geary, R.S. (2009). Antisense oligonucleotide pharmacokinetics and metabolism. Expet Opin. Drug Metabol. Toxicol. *5*, 381–391.

189. Krützfeldt, J., Rajewsky, N., Braich, R., Rajeev, K.G., Tuschl, T., Manoharan, M., and Stoffel, M. (2005). Silencing of microRNAs *in vivo* with 'antagomirs. Nature *438*, 685–689.

190. Amarzguioui, M., Holen, T., Babaie, E., and Prydz, H. (2003). Tolerance for mutations and chemical modifications in a siRNA. Nucleic Acids Res. *31*, 589–595.

191. Soutschek, J., Akinc, A., Bramlage, B., Charisse, K., Constien, R., Donoghue, M., Elbashir, S., Geick, A., Hadwiger, P., Harborth, J., et al. (2004). Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature *432*, 173–178.

192. Hall, A.H.S., Wan, J., Shaughnessy, E.E., Ramsay Shaw, B., and Alexander, K.A. (2004). RNA interference using boranophosphate siRNAs: structure-activity relationships. Nucleic Acids Res. *32*, 5991–6000.

193. Wang, J.-H., Gessler, D.J., Zhan, W., Gallagher, T.L., and Gao, G. (2024). Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct. Targeted Ther. *9*, 78.

194. Nathwani, A.C., Rosales, C., McIntosh, J., Rastegarlari, G., Nathwani, D., Raj, D., Nawathe, S., Waddington, S.N., Bronson, R., Jackson, S., et al. (2011). Long-term Safety and Efficacy Following Systemic Administration of a Self-complementary AAV Vector Encoding Human FIX Pseudotyped With Serotype 5 and 8 Capsid Proteins. Mol. Ther. *19*, 876–885.

195. Nathwani, A.C., Benjamin, R., Nienhuis, A.W., and Davidoff, A.M. (2004). Current status and prospects for gene therapy. Vox Sang. *87*, 73–81.

196. Pasi, K.J., Rangarajan, S., Mitchell, N., Lester, W., Symington, E., Madan, B., Laffan, M., Russell, C.B., Li, M., Pierce, G.F., and Wong, W.Y. (2020). Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A. N. Engl. J. Med. *382*, 29–40. https://doi.org/10.1056/NEJMoa1908490.

197. Davidsson, M., Negrini, M., Hauser, S., Svanbergsson, A., Lockowandt, M., Tomasello, G., Manfredsson, F.P., and Heuer, A. (2020). A comparison of AAV-vector production methods for gene therapy and preclinical assessment. Sci. Rep. *10*, 21532.

198. Smalley, E. (2017). First AAV gene therapy poised for landmark approval. Nat. Biotechnol. *35*, 998–999.

199. Jacobson, S.G., Boye, S.L., Aleman, T.S., Conlon, T.J., Zeiss, C.J., Roman, A.J., Cideciyan, A.V., Schwartz, S.B., Komaromy, A.M., Doobrajh, M., et al. (2006). Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in Leber congenital amaurosis. Hum. Gene Ther. *17*, 845–858.

200. Heo, Y.A. (2023). Etranacogene Dezaparvovec: First Approval. Drugs *83*, 347–352.

201. (2023). U.S. Food and Drug Administration*2024* (U.S. Food & Drug).

202. Frangoul, H., Locatelli, F., Sharma, A., Bhatia, M., Mapara, M., Molinari, L., Wall, D., Liem, R.I., Telfer, P., Shah, A.J., et al. (2024). Exagamglogene Autotemcel for Severe Sickle Cell Disease. N. Engl. J. Med. *390*, 1649–1662.

203. Aronson, S.J., Veron, P., Collaud, F., Hubert, A., Delahais, V., Honnet, G., de Knegt, R.J., Junge, N., Baumann, U., Di Giorgio, A., et al. (2019). Prevalence and Relevance of Pre-Existing Anti-Adeno-Associated Virus Immunity in the Context of Gene Therapy for Crigler-Najjar Syndrome. Hum. Gene Ther. *30*, 1297–1305.

204. Vandamme, C., Adjali, O., and Mingozzi, F. (2017). Unraveling the Complex Story of Immune Responses to AAV Vectors Trial After Trial. Hum. Gene Ther. *28*, 1061–1074.

205. Nayak, S., and Herzog, R.W. (2010). Progress and prospects: immune responses to viral vectors. Gene Ther. *17*, 295–304.

206. Sack, B.K., and Herzog, R.W. (2009). Evading the immune response upon *in vivo* gene therapy with viral vectors. Curr. Opin. Mol. Therapeut. *11*, 493–503.

207. Aix, E., Gutiérrez-Gutiérrez, Ó., Sánchez-Ferrer, C., Aguado, T., and Flores, I. (2016). Postnatal telomere dysfunction induces cardiomyocyte cell-cycle arrest through p21 activation. J. Cell Biol. *213*, 571–583.

208. Nguyen, G.N., Everett, J.K., Kafle, S., Roche, A.M., Raymond, H.E., Leiby, J., Wood, C., Assenmacher, C.-A., Merricks, E.P., Long, C.T., et al. (2021). A long-term study of AAV gene therapy in dogs with hemophilia A identifies clonal expansions of transduced liver cells. Nat. Biotechnol. *39*, 47–55.

209. Donahue, N.D., Acar, H., and Wilhelm, S. (2019). Concepts of nanoparticle cellular uptake, intracellular trafficking, and kinetics in nanomedicine. Adv. Drug Deliv. Rev. *143*, 68–96.

210. Rai, R., Alwani, S., and Badea, I. (2019). Polymeric Nanoparticles in Gene Therapy: New Avenues of Design and Optimization for Delivery Applications. Polymers *11*, 745.

211. Kamaly, N., Yameen, B., Wu, J., and Farokhzad, O.C. (2016). Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release. Chem. Rev. *116*, 2602–2663.

212. Crucho, C.I.C., and Barros, M.T. (2017). Polymeric nanoparticles: A study on the preparation variables and characterization methods. Mater. Sci. Eng., C *80*, 771–784.

213. Zhong, H., Chan, G., Hu, Y., Hu, H., and Ouyang, D. (2018). A Comprehensive Map of FDA-Approved Pharmaceutical Products. Pharmaceutics *10*, 263.

214. Xiao, B., Zhang, Z., Viennois, E., Kang, Y., Zhang, M., Han, M.K., Chen, J., and Merlin, D. (2016). Combination Therapy for Ulcerative Colitis: Orally Targeted Nanoparticles Prevent Mucosal Damage and Relieve Inflammation. Theranostics *6*, 2250–2266.

215. van den Berg, A.I.S., Yun, C.-O., Schiffelers, R.M., and Hennink, W.E. (2021). Polymeric delivery systems for nucleic acid therapeutics: Approaching the clinic. J. Contr. Release *331*, 121–141.

216. Akinc, A., Maier, M.A., Manoharan, M., Fitzgerald, K., Jayaraman, M., Barros, S., Ansell, S., Du, X., Hope, M.J., Madden, T.D., et al. (2019). The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nat. Nanotechnol. *14*, 1084–1087.

217. Vercauteren, D., Vandenbroucke, R.E., Jones, A.T., Rejman, J., Demeester, J., De Smedt, S.C., Sanders, N.N., and Braeckmans, K. (2010). The use of inhibitors to study endocytic pathways of gene carriers: optimization and pitfalls. Mol. Ther. *18*, 561–569.

218. Plank, C., Mechtler, K., Szoka, F.C., Jr., and Wagner, E. (1996). Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene delivery. Hum. Gene Ther. *7*, 1437–1446.

219. Breunig, M., Lungwitz, U., Liebl, R., and Goepferich, A. (2007). Breaking up the correlation between efficacy and toxicity for nonviral gene delivery. Proc. Natl. Acad. Sci. USA *104*, 14454–14459.

220. Ke, X., Shelton, L., Hu, Y., Zhu, Y., Chow, E., Tang, H., Santos, J.L., and Mao, H.-Q. (2020). Surface-Functionalized PEGylated Nanoparticles Deliver Messenger RNA to Pulmonary Immune Cells. ACS Appl. Mater. Interfaces *12*, 35835–35844.

221. Tan, L., Zheng, T., Li, M., Zhong, X., Tang, Y., Qin, M., and Sun, X. (2020). Optimization of an mRNA vaccine assisted with cyclodextrin-polyethyleneimine conjugates. Drug Deliv. Transl. Res. *10*, 678–689.

222. Kulkarni, J.A., Cullis, P.R., and van der Meel, R. (2018). Lipid Nanoparticles Enabling Gene Therapies: From Concepts to Clinical Utility. Nucleic Acid Therapeut. *28*, 146–157.

223. Love, K.T., Mahon, K.P., Levins, C.G., Whitehead, K.A., Querbes, W., Dorkin, J.R., Qin, J., Cantley, W., Qin, L.L., Racie, T., et al. (2010). Lipid-like materials for low-dose, *in vivo* gene silencing. Proc. Natl. Acad. Sci. USA *107*, 1864–1869.

224. Dong, Y., Love, K.T., Dorkin, J.R., Sirirungruang, S., Zhang, Y., Chen, D., Bogorad, R.L., Yin, H., Chen, Y., Vegas, A.J., et al. (2014). Lipopeptide nanoparticles for potent and selective siRNA delivery in rodents and nonhuman primates. Proc. Natl. Acad. Sci. USA *111*, 3955–3960.

225. Semple, S.C., Akinc, A., Chen, J., Sandhu, A.P., Mui, B.L., Cho, C.K., Sah, D.W.Y., Stebbing, D., Crosley, E.J., Yaworski, E., et al. (2010). Rational design of cationic lipids for siRNA delivery. Nat. Biotechnol. *28*, 172–176.

226. Adams, D., Gonzalez-Duarte, A., O'Riordan, W.D., Yang, C.-C., Ueda, M., Kristen, A.V., Tournev, I., Schmidt, H.H., Coelho, T., Berk, J.L., et al. (2018). Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N. Engl. J. Med. *379*, 11–21.

227. Rurik, J.G., Tombácz, I., Yadegari, A., Méndez Fernández, P.O., Shewale, S.V., Li, L., Kimura, T., Soliman, O.Y., Papp, T.E., Tam, Y.K., et al. (2022). CAR T cells produced *in vivo* to treat cardiac injury. Science *375*, 91–96.

228. Roberts, T.C., Langer, R., and Wood, M.J.A. (2020). Advances in oligonucleotide drug delivery. Nat. Rev. Drug Discov. *19*, 673–694.

229. Morell, A.G., Gregoriadis, G., Scheinberg, I.H., Hickman, J., and Ashwell, G. (1971). The role of sialic acid in determining the survival of glycoproteins in the circulation. J. Biol. Chem. *246*, 1461–1467.

230. Ashwell, G. (2008). sweet on science. Nat. Med. *14*, 608.

231. Fiume, L., Mattioli, A., Balboni, P.G., Tognon, M., Barbanti-Brodano, G., de Vries, J., and Wieland, T. (1979). Enhanced inhibition of virus DNA synthesis in hepatocytes by trifluorothymidine coupled to asialofetuin. FEBS Lett. *103*, 47–51.

232. Fiume, L., Mattioli, A., Busi, C., Balboni, P.G., Barbanti-Brodano, G., De Vries, J., Altmann, R., and Wieland, T. (1980). Selective inhibition of Ectromelia virus DNA synthesis in hepatocytes by adenine-9-beta-D-arabinofuranoside (ara-A) and adenine-9-beta-D-arabinofuranoside 5'-monophosphate (ara-AMP) conjugated to asialofetuin. FEBS Lett. *116*, 185–188.

233. Attie, A.D., Pittman, R.C., and Steinberg, D. (1980). Metabolism of native and of lactosylated human low density lipoprotein: evidence for two pathways for catabolism of exogenous proteins in rat hepatocytes. Proc. Natl. Acad. Sci. USA *77*, 5923–5927.

234. Nair, J.K., Willoughby, J.L.S., Chan, A., Charisse, K., Alam, M.R., Wang, Q., Hoekstra, M., Kandasamy, P., Kel'in, A.V., Milstein, S., et al. (2014). Multivalent N-Acetylgalactosamine-Conjugated siRNA Localizes in Hepatocytes and Elicits Robust RNAi-Mediated Gene Silencing. J. Am. Chem. Soc. *136*, 16958–16961.

235. Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., et al. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA *323*, 1061–1069.

236. (2024). World Health Organization*2024*.

237. Hou, X., Zaks, T., Langer, R., and Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. Nat. Rev. Mater. *6*, 1078–1094.

238. Jackson, L.A., Anderson, E.J., Rouphael, N.G., Roberts, P.C., Makhene, M., Coler, R.N., McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., et al. (2020). An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N. Engl. J. Med. *383*, 1920–1931.

239. (2023). World Health Organization.

240. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science *337*, 816–821.

241. Gillmore, J.D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M.L., Seitzer, J., O'Connell, D., Walsh, K.R., Wood, K., et al. (2021). CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. N. Engl. J. Med. *385*, 493–502.

242. McGuirk, J., Bachier, C.R., Bishop, M.R., Ho, P.J., Murthy, H.S., Dickinson, M.J., Maakaron, J.E., Andreadis, C., Ghobadi, A., Waller, E.K., et al. (2021). A phase 1 dose escalation and cohort expansion study of the safety and efficacy of allogeneic CRISPR-Cas9–engineered T cells (CTX110) in patients (Pts) with relapsed or refractory (R/R) B-cell malignancies (CARBON). J. Clin. Oncol. *39*, TPS7570.

243. Adams, D., Tournev, I.L., Taylor, M.S., Coelho, T., Planté-Bordeneuve, V., Berk, J.L., González-Duarte, A., Gillmore, J.D., Low, S.-C., Sekijima, Y., et al. (2023). Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial. Amyloid *30*, 1–9.

244. Khattak, A., Carlino, M., Meniawy, T., Ansstas, G., Medina, T., Taylor, M.H., Kim, K.B., McKean, M., Long, G.V., Sullivan, R.J., 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*, CT001.

245. Anttila, V., Saraste, A., Knuuti, J., Jaakkola, P., Hedman, M., Svedlund, S., Lagerström-Fermér, M., Kjaer, M., Jeppsson, A., and Gan, L.M. (2020). Synthetic mRNA Encoding VEGF-A in Patients Undergoing Coronary Artery Bypass Grafting: Design of a Phase 2a Clinical Trial. Mol. Ther. Methods Clin. Dev. *18*, 464–472.

246. Li, Y., Huo, Y., Yu, L., and Wang, J. (2019). Quality Control and Nonclinical Research on CAR-T Cell Products: General Principles and Key Issues. Engineering *5*, 122–131.

247. Rafiq, S., Hackett, C.S., and Brentjens, R.J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat. Rev. Clin. Oncol. *17*, 147–167.

248. Chen, X., and Gonçalves, M.A.F.V. (2016). Engineered Viruses as Genome Editing Devices. Mol. Ther. *24*, 447–457.

249. Qiu, M., Glass, Z., Chen, J., Haas, M., Jin, X., Zhao, X., Rui, X., Ye, Z., Li, Y., Zhang, F., and Xu, Q. (2021). Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific *in vivo* genome editing of *Angptl3*. Proc. Natl. Acad. Sci. USA *118*, e2020401118.

250. Gillmore, J.D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M.L., Seitzer, J., O'Connell, D., Walsh, K.R., Wood, K., et al. (2021). CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. N. Engl. J. Med. *385*, 493–502.

251. De Velasco, M.A., Kura, Y., Sakai, K., Hatanaka, Y., Davies, B.R., Campbell, H., Klein, S., Kim, Y., MacLeod, A.R., Sugimoto, K., et al. (2019). Targeting castration-resistant prostate cancer with androgen receptor antisense oligonucleotide therapy. JCI insight *4*, e122688.

252. Laskin, J.J., Nicholas, G., Lee, C., Gitlitz, B., Vincent, M., Cormier, Y., Stephenson, J., Ung, Y., Sanborn, R., Pressnail, B., et al. (2012). Phase I/II Trial of Custirsen (OGX-011), an Inhibitor of Clusterin, in Combination with a Gemcitabine and Platinum Regimen in Patients with Previously Untreated Advanced Non-small Cell Lung Cancer. J. Thorac. Oncol. *7*, 579–586.

253. Crooke, S.T., Liang, X.-H., Baker, B.F., and Crooke, R.M. (2021). Antisense technology: A review. J. Biol. Chem. *296*, 100416.

254. Swayze, E.E., and Bhat, B. (2007). The medicinal chemistry of oligonucleotides. In Antisense Drug Technology (CRC Press), pp. 161–200.

255. Koch, T., and Ørum, H. (2007). Locked nucleic acid. In Antisense Drug Technology (CRC Press), pp. 537–582.

256. Anderson, B.A., Freestone, G.C., Low, A., De-Hoyos, C.L., Iii, W.J.D., Østergaard, M.E., Migawa, M.T., Fazio, M., Wan, W.B., Berdeja, A., et al. (2021). Towards next generation antisense oligonucleotides: mesylphosphoramidate modification improves therapeutic index and duration of effect of gapmer antisense oligonucleotides. Nucleic Acids Res. *49*, 9026–9041.

257. Ray, K.K., Landmesser, U., Leiter, L.A., Kallend, D., Dufour, R., Karakas, M., Hall, T., Troquay, R.P.T., Turner, T., Visseren, F.L.J., et al. (2017). Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol. N. Engl. J. Med. *376*, 1430–1440.

258. Hoppe, B., Koch, A., Cochat, P., Garrelfs, S.F., Baum, M.A., Groothoff, J.W., Lipkin, G., Coenen, M., Schalk, G., Amrite, A., et al. (2022). Safety, pharmacodynamics, and exposure-response modeling results from a first-in-human phase 1 study of nedosiran (PHYOX1) in primary hyperoxaluria. Kidney Int. *101*, 626–634.