The growing understanding of RNA functions and their crucial roles in diseases promotes the application of various RNAs to selectively function on hitherto “undruggable” proteins, transcripts and genes, thus potentially broadening the therapeutic targets. Several RNA-based medications have been approved for clinical use, while others are still under investigation or preclinical trials. Various techniques have been explored to promote RNA intracellular trafficking and metabolic stability, despite significant challenges in developing RNA-based therapeutics. In this review, the mechanisms of action, challenges, solutions, and clinical application of RNA-based therapeutics have been comprehensively summarized.

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**FACTS**

- Small molecules and antibody drugs target only 0.05% of the human genome, and most disease targets lack defining active sites for small-molecule binding.
- Abundant RNAs selectively act on proteins, transcripts and genes, which broaden the range of druggable targets. A defined sequence of RNA drugs makes the design of RNA therapeutics much easier.
- Multiple RNA drugs are approved and a dozen more are in phase III trials to treat rare and common diseases.

**OPEN QUESTIONS**

- How do RNA molecules function in treating diseases?
- The challenges and solutions to the discovery and development of RNA-based drugs effectively.

**INTRODUCTION**

The antisense oligonucleotides (ASOs) inhibiting protein synthesis in the early 1980s promoted the rapid advances of RNA-based therapeutics [1]. In the 2000s, the proposal of RNAi and the use of siRNA silencing the human gene led to increased funding for RNA therapeutics [2]. Other RNA molecule regulators and related mechanisms also have been well characterized. So far, there are several approved RNA-based medications and many in phase III studies [3] (Fig. 1).

Compared to conventional protein-targeted and DNA-based medicines, RNA-based therapeutics are prospective due to their distinct physicochemical and physiological properties [4]. RNAs function in three essential biological macromolecules: DNAs, RNAs, and proteins. RNA molecules such as ASOs, small interfering RNA (siRNAs), and microRNAs (miRNAs) can directly target mRNAs and noncoding RNAs (ncRNAs) through Watson–Crick base-pairing [4]. Therefore, RNA can theoretically target any interest gene by selecting the correct nucleotide sequence on the target RNA. By contrast, only 0.05% of the human genome has been drugged by the currently approved protein-targeted therapeutics (small-molecule chemicals and antibodies) since most DNA sequences of the human genome are transcribed into noncoding transcripts [5]. Besides, around 85% of proteins lack specific clefts and pockets for small molecules binding [6]. In addition, in vitro transcribed (IVT) mRNA can be applied for protein replacement treatment or immunization after entering the cytoplasm [7]. This process would not cause irreversible genome changes and induce genetic risks like DNA-based therapeutics [8]. Moreover, clustered regularly interspaced short palindromic repeat (CRISPR)-based genome editing can directly modify target RNA sequences to treat specific disorders [9]. RNA aptamers can also block protein activity, similar to small-molecule inhibitors and antibodies [10]. Therefore, RNA-based therapies can broaden the range of druggable targets and are regarded as the most attractive therapeutic target.

This review provides an overview of significant developments in RNA-based therapeutics. The classification of RNA-based therapies and their modes of action have been outlined. This review also covers critical challenges in applying these RNA therapies and possible solutions. Finally, the current preclinical and clinical trials of RNA therapeutics have also been summarized.

**TYPES OF RNA-BASED THERAPEUTICS AND MODES OF ACTION**

**Antisense oligonucleotides (ASOs)**

The ASOs are short single-stranded (ss) oligonucleotides (12–24 nucleotides (nt)) complementary to the specific RNA through
ASOs can also bind to inhibitory elements, such as upstream open reading frames (uORFs) [23] or other translation inhibitory components (TIEs) [24], thus upregulating target RNAs (Fig. 2A). Furthermore, ASOs can inhibit miRNA-mediated downregulation by directly binding to miRNAs (miRNA inhibitors or antagomirs) [25] or combining with miRNAs at miRNA-binding sites to inhibit miRNA interaction (miRNA competitors or block-mir) [26] (Fig. 2A). Occupancy-only ASOs allow more nucleotide modifications than RNase H1-dependent ASOs, thus improving ASO drug properties. Notably, occupancy-only ASOs should not form RNA-DNA duplex to avoid forming RNase H1or Ago2 substrates and unnecessary cleavage of target RNA.

**RNA interference (RNAi)**

RNAi is an endogenous cellular process inducing double-stranded (ds) RNAs -triggered degradation of particular RNA targets, which provide an intrinsic defensive mechanism against invading viruses and transposable elements [2, 27, 28]. siRNAs are short dsRNAs (20-24 nt) with distinct structures containing 5′-phosphate/3′-hydroxyl endings and two 3′-overhang ribonucleotides on each duplex strand [29, 30]. siRNAs can induce RNAi in mammalian cells. Therefore, researchers can use such simple gene silencing tools to investigate gene function and advance disease therapy [31]. Mechanistically, the endoribonuclease Dicer cuts dsRNAs and isolates the guide and passenger strands within the RNA-induced silencing complex (RISC). The argonaeute2 (AGO2) protein degrades the passenger siRNA strand, whereas the guide siRNA strand directly binds to the target RNA, causing AGO2-mediated cleavage [32] (Fig. 2B). Besides degrading cytoplasmic RNAs, siRNAs can also trigger chromatin remodeling and histone modifications in the nucleus when they bind to the promoter regions, resulting in transcriptional silence [31, 33].

miRNAs are endogenous single-stranded small ncRNAs influencing gene expression via RNAi [34]. The biogenesis of miRNAs follows a systematic process. First, miRNAs are produced from lengthy primary precursor miRNAs (pri-miRNAs), cleaved in the nucleus by RNase III-family nuclease Drosha. The free pre-miRNAs are then transported to the cytoplasm, where their loop regions are cleaved by Dicer to produce mature miRNA. The mature miRNA duplex (comprising two strands) is loaded into the pre-RISC for strand selection. One of the two miRNA strands is selectivity loaded into the miRNA-induced silencing complex (miRISC), whereas the other strand is ejected from the complex and is subject to degradation. Both strands may be loaded into RISC at similar frequencies, while different strand usage depending on biological contexts. The strand from the 5′ end of
the stem-loop and the 3′ strand are named “5p” and “3p”, respectively [35, 36]. Finally, miRNAs cause mRNA translational repression or degradation in the miRNA-induced silencing complex (miRISC) by base-pairing with specific RNA sequences (often in the 3′ untranslated region (UTR)) [34] (Fig. 2B). Interestingly, emerging evidence has revealed that miRNAs can upregulate targeted genes through increasing mRNA stability or/and translation [37]. For example, miR-346 targets the amyloid-β peptide precursor protein (APP) mRNA 5′-UTR to upregulate APP translation and amyloid-β production [38]. miR-466l elevates IL-10 mRNA stability and IL-10 protein expression through binding to IL-10 AU-rich elements [39]. miRNAs have crucial roles in regulating gene expression and human diseases. miRNA mimics have been currently in preclinical development as putative therapeutic agents. Chemically modified, completely base-paired siRNAs with the identical guide strand sequence as an endogenous miRNA are widely used as miRNA mimics [40].

Unlike RNAi, RNA activation (RNAa) is a process where dsRNA triggers gene production by targeting promoter sequences [41]. Small activating RNAs (saRNAs) are synthesized using homologous sequences close or within gene promoters, which can trigger RNAa. Similar to miRNA-like target recognition, saRNAs actions depend on
the AGO2 protein. In the nucleus, AGO2–saRNA uses the “seed” region to basepair with sequences inside the chromatin-bound RNA transcripts or complementary DNA [41–43]. Besides saRNA and AGO2, recent research found that RNA-induced transcriptional activation (RITA) complex also contains RHA and CTR9 [44]. saRNA AGO2, recent research found that RNA-induced transcriptional activation (RITA) complex also contains RHA and CTR9 [44]. saRNA can alleviate the downregulation of silent tumor suppressor genes or knockin of a target gene [46]. Mechanistically, this system relies on a designed guide RNA (gRNA) and an RNA-guided Cas nuclease. The gRNA forms the Cas-gRNA ribonucleoprotein complex by binding to Cas. The complex recognizes a protospacer-adjacent motif (PAM) element and a 20-nucleotide sequence in the target sequence. The Cas nuclease then cleaves the dsDNA or ssRNA at the specific site for efficient genome editing [47]. Initial successes have expanded the development of new methods for targeting and manipulating nucleic acids, such as Cas9 and Cas13 orthologues-derived methods [9]. The CRISPR/Cas-based RNA editing system includes two Cas nuclease categories, Cas9 and Cas13. A guide RNA (gRNA) binds to Cas9 to cleave ssRNA with (①) or without (②) the help of a protospacer-adjacent motif (PAM). ① A single CRISPR RNA (crRNA) guides Cas13 to target specific RNA having a protospacer flanking sequence (PFS). In addition to knockdown target RNA, a catalytically deactivated Cas13b (dCas9b) facilitates the A-to-I editing with ADAR. ② RNA aptamers function as agonists or delivery agents. ② As an antagonist aptamer, Pegaptanib interacts specifically with vascular endothelial growth factor (VEGF) to inhibit the recruitment of VEGF with its receptors, thus treating macular degeneration. ③ Cell type-specific RNA aptamers deliver agents (mRNA, siRNA, shRNA, antibody and chemotherapy drugs) by binding to or conjugating. ③ mRNA vaccine. ③ The mRNA vaccine against SARS-CoV-2 (mRNA-1273) is delivered into antigen-presenting cells by lipid nanoparticle (LNP). ③ The mRNA encoding SARS-CoV-2 spike protein is released into the cytoplasm and translated to antigen protein by the ribosome. ③ Some antigen proteins are degraded into small peptides by the proteasome and presented to the surface of CD8+ T cells by major histocompatibility complex I (MHC-I). The CD8+ cytotoxic T-cell-mediated immunity kills infected cells by secreting perforin or granzyme. ③ Other antigen proteins are degraded in the lysosome and displayed on the surface of T helper cells by MHC II. The B-cell/antibody-mediated humoral immune uses antibodies to neutralize pathogens.

CRISPR-based genome editing

The protokaryote-derived CRISPR-associated protein (Cas) systems have been widely used in mammalian cells and organisms to precisely edit genome sequence, resulting in irreversible knockout or knockin of a target gene [46]. Mechanistically, this system relies on a designed guide RNA (gRNA) and an RNA-guided Cas nuclease. The gRNA forms the Cas-gRNA ribonucleoprotein complex by binding to Cas. The complex recognizes a protospacer-adjacent motif (PAM) element and a 20-nucleotide sequence in the target sequence. The Cas nuclease then cleaves the dsDNA or an ssRNA at the specific site for efficient genome editing [47]. Initial successes have expanded the development of new methods for targeting and manipulating nucleic acids, such as Cas9 and Cas13 orthologues-derived methods [9]. The Cas9 system can target both dsDNA and ssRNA. The Cas9 target RNA of Streptococcus pyogenes (RCas9) requires a matching gRNA and complementary PAM-presenting oligonucleotide (PAMmer) [48] (Fig. 2C①). Cas9 orthologs (Cas9 of Campylobacter jejuni and Staphylococcus aureus) can cleave ssRNA without PAM [49] (Fig. 2C②). Cas13-mediated systems only target RNA, where a CRISPR RNA (crRNA) guides Cas13 to cleave specific RNA. Cas13a, Cas13b and Cas13d have been verified to interfere with and silence target RNA in mammal cells in vitro. CasRx (RfxCas13d), a subtype of Cas13d, showed the most potent RNA knockdown efficiency in HEK293T cells [50]. A protospacer flanking sequence (PFS) may be required to cut the target and non-target RNA molecules via two conserved Higher Eukaryotes and Prokaryotes Nucleotide-binding (HEPN) domains [51] (Fig. 2C③). Cas13d is a new PFS-independent Cas, and a non-catalytic variant of Cas13b that lacks endonuclease activity can induce the A-to-I base switch by fusing with the ADAR2 deaminase domain (ADAR2ΔD) [52] (Fig. 2C④). A Antisense oligonucleotides (ASOs) can modulate the target gene expression through two mechanisms. (①) In the occupancy-mediated degradation way, ASOs trigger the target mRNA cleavage by RNase H1 or ribozymes. The Occupancy-only mechanisms do not directly degrade target RNA. Instead, it regulates the gene expression in several ways: (②) alter RNA splicing using splice-switching ASOs to induce exon skipping or exon inclusion; (③) lead to nonsense-mediated mRNA decay (NMD); (④) inhibit or activate translation; (⑤) block the microRNAs binding to target mRNA. B RNA interference (RNAi). Long double-stranded RNA (dsRNA) and precursor microRNA (pre-miRNA) are processed by Dicer into short interfering RNA (siRNA). The antisense strand (indicated as a blue strand) of siRNA is loaded into the RNA-induced silencing complex (RISC) for RNA targeting, degradation or translation repression.

C CRISPR/Cas-based RNA editing system includes two Cas nuclease categories, Cas9 and Cas13. A guide RNA (gRNA) binds to Cas9 to cleave ssRNA with (①) or without (②) the help of a protospacer-adjacent motif (PAM). ① A single CRISPR RNA (crRNA) guides Cas13 to target specific RNA having a protospacer flanking sequence (PFS). In addition to knockdown target RNA, a catalytically deactivated Cas13b (dCas9b) facilitates the A-to-I editing with ADAR. ② RNA aptamers function as agonists or delivery agents. ② As an antagonist aptamer, Pegaptanib interacts specifically with vascular endothelial growth factor (VEGF) to inhibit the recruitment of VEGF with its receptors, thus treating macular degeneration. ③ Cell type-specific RNA aptamers deliver agents (mRNA, siRNA, shRNA, antibody and chemotherapy drugs) by binding to or conjugating. ③ mRNA vaccine. ③ The mRNA vaccine against SARS-CoV-2 (mRNA-1273) is delivered into antigen-presenting cells by lipid nanoparticle (LNP). ③ The mRNA encoding SARS-CoV-2 spike protein is released into the cytoplasm and translated to antigen protein by the ribosome. ③ Some antigen proteins are degraded into small peptides by the proteasome and presented to the surface of CD8+ T cells by major histocompatibility complex I (MHC-I). The CD8+ cytotoxic T-cell-mediated immunity kills infected cells by secreting perforin or granzyme. ③ Other antigen proteins are degraded in the lysosome and displayed on the surface of T helper cells by MHC II. The B-cell/antibody-mediated humoral immune uses antibodies to neutralize pathogens.

mRNAs and mRNA vaccine

The concept of mRNA-encoded drugs was discovered in the 1990s when direct injection of IVT mRNA into the mouse skeletal muscle showed encoded protein expression [56]. Preclinical research on IVT mRNA promotes the clinical development of mRNA-based vaccination against cancer and infectious disease [7]. Mechanistically, injected mRNA vaccines are delivered into the cytoplasm of the host cell (typically antigen-presenting cells (APCs)) and are translated into the targeted antigens. Subsequently, the major histocompatibility complexes (MHCs) present the expressed antigens to the surface of APCs to activate B cell/antibody-mediated humoral immunity and CD4+ T/CD8+ cytotoxic T-cell-mediated immunity [57]. Besides, injected mRNA encoding immunostimulants (cytokines, chemokines, etc.) can promote APC maturation and activation, thus inducing a T-cell-mediated response and improving the immune tumor microenvironment [58] (Fig. 2E).

OVERCOMING CHALLENGES IN THE DEVELOPMENT OF RNA THERAPEUTICS

The efficiency of RNA delivery into the cytoplasm through overcoming the extracellular and intracellular barriers remains critical for successful RNA therapy. Firstly, large, hydrophilic, negatively-charged properties prevent RNA from passively diffusing across the lipid bilayers. Beyond the physical barrier, RNA drugs must evade serum nucleases and scavenge macrophages within the reticuloendothelial system. Moreover, RNA drugs must pass through the extracellular matrix and across the cell membrane through receptor-mediated endocytosis. Escaping from the endosome and releasing RNAs into the cytoplasm in a non-toxic manner is a critical technical problem [59]. Therefore, various chemical modifications [60, 61] and the engineering of delivery formulations [62, 63] have been explored to solve challenges related to pharmacodynamics and pharmacokinetics.

CHEMICAL MODIFICATIONS

Chemical modifications of ASOs and siRNAs

Chemical modification of ASOs and siRNAs
nuclease resistance and delivery. Besides, it reduces toxicity and immunogenicity [64]. The modification of phosphorothioate (PS) backbones (Fig. 3A) was the first and most commonly used chemical modification in ASOs. In this modification, one of the non-bridging oxygen atoms in the inter-nucleotide phosphate group is replaced with sulfur [65]. The PS backbone modification can facilitate cellular uptake and bioavailability in vivo via increased hydrophobicity, resistance to phosphodiesterases and avidly binding serum proteins [65]. The modifications of 2′ sugar at the ribose, including 2′-fluoro (F), 2′-methoxyethyl (MOE), 2′-O-methyl (O-Me) or 2′,4′-bicyclics with O-methylene bridge or locked nucleic acid (LNA), can improve the binding affinity and increase base-pairing melting temperature [66, 67] (Fig. 3A). The 2′-F and 2′-O-Me modifications imitate the biophysical features of 2′-OH and can stabilize siRNAs against RNases while also preventing siRNAs activating innate immune receptors (TLR, MDA-5, and RIG-I). As a result, all therapeutic siRNAs in clinical trials have 2′-F or 2′-O-Me modifications [68]. LNA and its methylated derivative (“constrained ethyl” (cEt)) are widely used in ASOs, including gapmers, split-switching ASOs, siLNA and antegene ASOs. These chimeric LNA or DNA oligonucleotides are more stable than isosequential PSs and 2′-O-Me gapmers [69]. Many 5′- or 3′-RNA conjugates (folate and N-acetylgalactosamine (GalNAc)) can also improve delivery. GalNAc is a high-affinity ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR), thereby significantly enhancing ASO and siRNA delivery to the liver [70] (Fig. 3A). Phosphorodiamidate morpholino oligonucleotide (PMO) [71] and peptide nucleic acid (PNA) [72] are more complex modifications that entirely alter the linking moieties while maintaining nucleobases for pairing (Fig. 3A).

**Chemical modifications of mRNA, CRISPR-Cas guide RNAs, and aptamer**

IVT mRNA is an ssRNA containing a 5′ cap structure, an ORF, flanking 5′ and 3′ UTRs and a 3′ poly (A) tail [73]. The optimization of mRNA vaccines often starts from these five structures through sequence optimization, nucleoside modification or sequence substitution of UTRs to enhance RNAs’ translational ability [74]. Various chemical modification nucleosides, including pseudouridine (Ψ), N1-methylpseudouridine (m1Ψ), 5-methylcytidine (m5C), 5-hydroxymethylcytosine (5hmC), 5-methyluridine (m5U) and 2-thiouridine (s2U) have been introduced into mRNA to minimize detectable off-target effects, potential immunogenicity, etc. Chemical modification patterns used in ASOs and siRNAs can be applied to gRNA and Cas9 mRNA [77]. For instance, chemical modifications containing 2′-O-M-3′PS (MS), 2′-O-M, or 2′-OM 3′-thioPACE (MSP) can be integrated into single-gRNAs (sgRNAs) at three terminal nucleotides on both the 5′ and 3′ ends to improve genome editing efficiency in human primary T cells and CD34+ hematopoietic stem and progenitor cells [78]. The ribose-phosphate backbone of gRNAs with 2′-OM-3′-phosphonoacetate (MP) modification can significantly minimize off-target cleavage while maintaining strong on-target performance [79]. The 5′-H group modification of sgRNAs can be highly active and evade innate immune responses [80]. Incorporating bridging nucleic acids (2′,4′-BNANC[N-Me]) and LNA at particular sites in crRNA can significantly reduce off-target DNA cleavage by Cas9 [81]. The chemical modification of IVT mRNA can be used on Cas9 mRNA. Like ASOs and siRNAs, versatile chemical modifications and conjugations can enhance the pharmacokinetic features of RNA aptamers [82]. Most aptamers in the clinical trials are chemically modified using 5′-end polyethylene glycol (PEG)ylation (for resisting renal clearance) [83], 3′-end-capping strategy with inverted thymidine [84], and 2′-substitutions on the sugar ring (for preventing nucleotide degradation). PS linkage modification can also optimize the properties of aptamers by improving target binding affinity [85].

**DELIVERY FORMULATION WITH NANOCARRIERS**

Developing an effective carrier to protect the carried RNA from the harmful physiological environment is necessary since RNAs have substantial negative charges and chemical modifications.
Advances in nanotechnology and materials science offer advantages and potential solutions to the challenges of oligonucleotide drug delivery, especially for the requirements of intracellular delivery across biological barriers and membranes. Key benefits of nanoparticle drug delivery systems include custom optimization of nanoparticle biophysics (e.g., size, shape, and chemical/material composition) and biological properties (e.g., targeting ligand functionalization), allowing for a high degree of customization delivery platform. Here we introduced two dominant delivery approaches—lipid nanoparticles and cationic polymer-based polyplexes, and three emerging novel nanocarriers. We summarize the representation of RNA therapeutics delivery as shown in Table 1.

**Lipid nanoparticles (LNPs)**

LNPs are the most widely used carriers to deliver oligonucleotide drugs [86, 87]. LNPs consist of ionizable cationic lipids, cholesterol, phospholipids and PEG-lipids (Fig. 3B). Ionizable cationic lipids are the core components. Cationic lipids form “lipoplexes” by electrostatically binding to negatively-charged nucleic acids, which have been widely used in vitro for nucleic acid transfections (e.g., Lipofectamine™ RNAiMAX transfection reagents). Helper lipids, phospholipids and cholesterol promote formulation stability and delivery efficiency [88]. PEG-lipid can control particle size, prevent particle aggregation, and lengthen in vivo circulation lifetimes [89]. Other reviews have comprehensively discussed the advanced formulation of LNPs’ optimization characteristics and production methods [90].

Using the LNP-mediated siRNA delivery as an example [91] (Fig. 3B). The acid dissociation constant (pKa) determines the nanoparticles’ ionization behavior and surface charge, thereby influencing the delivery process. Firstly, positively charged LNPs prevent anionic RNAs from nucleases by coating RNAs and help RNAs across the cell membrane through receptor-mediated endocytosis. After entering the cells, the charges on the nanoparticle increase as the pH decrease (from 7 to 5.5) during endosomal maturation. Nanoparticles with a pKa in this range are protonated and create a buffering capacity. The buffering capacity of nanoparticles and/or membrane destabilization causes osmotic swelling and endosome breaking. The charges on nanoparticles decrease in the cytosol and weaken the binding to siRNAs [92]. The siRNAs then escape from endosomes into the cytosol, the critical rate-limiting step for its delivery. As a result, siRNAs cleave target RNAs by associating with the RISC. Other RNA entities may be translated to proteins or translocated in the nucleus. Therefore, optimizing LNPs’ pKa dramatically increases the delivery efficiency of RNA drugs. Besides, LNP-loaded RNA entities may be safely and effectively delivered to specific cells, organs, and tissues using the emerging advanced technologies. The branched-tail LNP can sufficiently co-deliver three distinct mRNAs in vivo and are not immunogenic or toxic to the liver [93]. The engineered-ionizable LNP has been developed for selective delivery of RNA into various liver cells [94]. The selective organ targeting (SORT) technique could specifically target the liver and extrahepatic tissues (lung and spleen) by adding a SORT molecule into LNP. This technique enables mRNA delivery and CRISPR-Cas gene editing in specific tissues [95]. Therefore, LNP-based gene therapies can treat hepatic diseases and other rare diseases.

**Cationic polymer-based polyplexes**

Polyplexes are standard formulations used for nucleic acid delivery. They are spontaneously formed by electrostatic interactions between cationic polymers and negatively-charged nucleic acids (Fig. 3B). The polyethyleneimine (PEI) polymer family is the most widely studied polymeric material for nucleic acid delivery. They consist of linear or branched polycations that can form nanoscale complexes with miRNA or siRNA, thus leading to RNA protection and cellular delivery. A commercially available linear PEI derivative, jetPEI™, is widely used for DNA, siRNA and mRNA transfection. Besides, transforming RNA vaccines from PEI functionalized graphene oxide hydrogel in situ has been used for cancer immunotherapy effectively [96]. Targeted CRM197-PEG-PEI-based complexes for siRNA delivery in vivo show therapeutic effects by knockdown of growth factor pleiotrophin in glioblastoma [97]. However, PEI is relatively cytotoxic and not degradable [98]. Chitosan is a biopolymer found in the exoskeleton of crustaceans. Chitosan and its variants have been developed for DNA and siRNA delivery due to their biodegradability, biocompatibility and permeability-enhancing properties. However, the low transfection efficiency limits their clinical application [99]. Chitosan-grafted PEI has been synthesized with increased transfection efficiency and lower toxicity [98]. Cyclo-dextrin polymer (CDP)-based nanoparticles were the first targeted nanoparticle siRNA delivery system, which enters clinical trials for cancer. The self-assembling, CDP-based nanoparticle (denoted as CALAA-01) targets the ribonucleotide reductase M2 mRNA in patients with solid cancers [100, 101]. Other polymers like poly(β-amino esters) (PBAEs) [102] and polyaspartamides [103] are also used in nucleic acid delivery. However, there have been no polypeoxles nanoparticles for RNA delivery approved. Therefore, improved strategies are required for producing scalable cationic polymer-based polyplexes with high target specificity and less toxic or immunogenic.

**Emerging novel approaches—exosomes, spherical nucleic acids (SNAs), and DNA nanostructures**

Exosomes are lipid membrane-enclosed vesicles with a diameter ranging from 40 to 160 nm (Fig. 3B). They are found in all body fluids and are secreted by most cells. Exosomes are crucial intercellular communication mediators influencing many cell biology processes [104]. They can transport bioactive constituents and overcome biological barriers (blood–brain barrier). As a result, exosomes have attracted much attention as potential delivery vehicles for therapeutic agents. Engineered exosomes can deliver different RNA species with minimal immune clearance and adverse effects [105, 106].

SNAs are densely packed, radially oriented nucleic acids in the outer layer and covalently bond to an inorganic nanoparticle core. Gold nanoparticles (10–15 nm) are the most commonly used SNA cores. Alkyl thiol or cyclic disulfide chemical tethering groups are used to attach nucleic acids to the core surface. A recognition sequence (15–25 nt) is complementary to the target sequence, and a spacer (10 nt) connects the nanoparticle surface with the recognition sequence [107] (Fig. 3B). SNAs can effectively deliver nucleic acid because: (1) they show rapid cellular uptake kinetics and intracellular transport. Their 3D structure can be recognized by class A scavenger receptors. Then, the proximity of SNAs, class A scavenger receptors and lipid-raft microdomains allow SNAs to be rapidly internalized through endocytosis in a lipid-raft-dependent and caveolae-mediated manner [108]. (2) They induce a negligible immune response. The high density of nucleic acids at the surface of SNAs inhibits nucleases-mediated degradation. Therefore, they can enter almost any cell type and various biological barriers (skin, tumor, and blood–brain barrier) without using transfection agents. Delivery of SNAs by exosomes, modification of the oligonucleotide sugar backbone or PEGylation of nanoparticles could further enhance the effectiveness of SNAs [107]. Excilure, Inc. has developed SNA therapeutic platforms with four SNA-loaded drugs currently in clinical trials. Systematic delivery of SNAs carrying Bcl2Like12 (Bcl2L12) siRNA (NU-0129) can act as a brain-penetrant precision therapeutic strategy for intracerebral glioblastoma multiforme treatment [109]. AST-005 can treat psoriasi lesions by inhibiting TNF-α mRNA via ASOs. XCUR17 can target interleukin-17 receptor-α through ASOs for treating psoriasis micro plaques. ST-008, TLR9 agonist SNA, combined

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| Delivery method | Composition | Payload | Example | Advantage | Limitation | References |
|-----------------|-------------|---------|---------|-----------|------------|------------|
| Lipid nanoparticles | DLin-MC3-DMA (MC3) | siRNA | siRNA drug (Patisiran) | Increased half life; protection from nucleases acids | Elevated risk of immunotoxicity and immunogenicity | [86-89, 93-95, 147, 148] |
| ALC-0315 | mRNA, gRNA | SARS-CoV-2 mRNA vaccine (BNT162b2) | Facilitate the endosomal escape of RNA molecules; increase in protein expression or immune responses in mice compared to MC3 | | | |
| SM-102 | donor RNA | SARS-CoV-2 mRNA vaccine (mRNA-1273) | | | | |
| Cationic polymer-based polyplexes | Polyethyleneimine (PEI) | mRNA, siRNA, miRNA, gRNA | SiG1 2D LODER; CRM197-PEG-PEI-based complexes | High charge density and pH buffer capacity | Potential toxicity and plasma instability | [96-98, 136] |
| Chitosan | mRNA, siRNA, (DNA) | siRNA loaded chitosan lactate nanoparticles (CL-TAT-HA) | Low toxicity, biodegradability, biocompatibility, and permeability-enhancing | Low transfection efficiency | | [99] |
| Cyclodextrin polymer (CDP) | siRNA | CALAA-01 | | | | [100, 101] |
| Poly[(β-amino esters) (PBAEs)] | mRNA, gRNA, donor RNA | HPV16 E7-targeting CRISPR/short hairpin RNA (shRNA) | Low toxicity | Low charge density | | [102] |
| Exosomes | Derived sources: mesenchymal stem cells | miRNA, siRNA, mRNA, other ncRNAs | miR-124; KRAS G12D siRNA | With minimal immune clearance and adverse effects | | [105, 106] |
| Spherical nucleic acids | AuNP, quantum dots (QDs), SiO2, Ag, Fe3O4, | mRNA, siRNA, gRNA, donor RNA | NU-0129 (Bcl2Like12 (Bcl2L12) siRNA) | Show rapid cellular uptake kinetics and intracellular transport; induce a negligible immune response. | | [107, 109, 110] |
| DNA nanostructures | ASOs, siRNAs, aptamers, CRISPR-Cas9 | AS1411 aptamers | The object's size, shape and plasticity can be fine-tuned | Enzymatic hydrolysis, low cellular uptake, immune cell recognition and degradation, and unclear biodistribution profiles | | [111-114, 117] |
with immune checkpoint inhibitors, are used for immuno-oncology treatments [110].

DNA-based nanostructures are also widely used as delivery vehicles (Fig. 3B(3)). The self-assembly features of DNA allow it to fold into user-defined shapes through sequence-complementary domain hybridization. As a result, the object’s size can be precisely controlled at the nanometer scale, and the object’s shape and plasticity can be fine-tuned [111]. DNA nanostructures deliver RNA by incorporating ASOs or siRNAs on the structure surface [112]. AS1411 aptamers can also be inserted into DNA pyramids to promote intracellular uptake and nuclease degradation resistance [113]. The yarn-like DNA nanoclew can deliver CRISPR-Cas9 through rolling circle amplification and base-pairing with the Cas9/sRNA complex [114]. Moreover, cytosine-phosphate-guanine (CpG) oligonucleotides can target tissue macrophages when incorporated into DNA nanotubes by enhancing macrophage immunostimulation [115, 116]. However, the high costs of DNA and the high error rate of self-assembly limit the application of DNA nanostructures in emerging complex structures [117].

**APPLICATION**

RNA-based therapeutics were firstly adopted for diseases with clear rationales, such as oncology, neurological disorders and infectious diseases. Recent advancements in RNA technologies have led to the approval of eight ASOs, three siRNAs, and two mRNA vaccines for COVID-19 under the Emergency Use Authorization scheme (Table 2). Besides, other medicines are currently undergoing preclinical or clinical trials. Moreover, more advanced RNA-based therapeutics have been developed recently.

**Performance of ASOs in clinical trials**

Out of the 100 phase I trials performed on ASO-based therapies, a quarter has entered phase II/III trials in the last 5 years for treating rare and common diseases, such as orphan genetic alterations and cancer [11].

Fomivirsen is the first FDA-approved ASO drug for treating cytomegalovirus retinitis (CMV) in patients with AIDS [118]. This PS ASO complementary to human CMV immediate-early mRNA inhibits viral protein synthesis and interrupts viral replication [119]. However, since the introduction of highly active anti-retroviral therapy, cases of CMV have significantly reduced. Therefore, fomivirsen was withdrawn from the market in Europe and USA in 2002. Mipomersen and inotersen are second-generation ASO drugs called “Gapmers” or chimeric ASOs. Mipomersen effectively degrades apolipoprotein B (ApoB) mRNA. Likewise, inotersen mediates RNase H1-mediated degradation of hepatic transthyretin (TTR) mRNAs to reduce TTR protein synthesis and serum TTR levels [120]. Two splice-modulating ASOs (nusinersen and etepiliren) were approved in 2016 for treating splicing defects [121]. Nusinersen is the first splicing-correcting ASO approved for spinal muscular atrophy (SMA) treatment. The 2′-MOE ASO promotes exon 7 inclusion and increases the expression of SMN protein by binding to the intronic splicing inhibitor in intron 7 [18]. Etepliren is another splice-modulating oligonucleotide used to treat Duchenne muscular dystrophy (DMD) patients. It hybridizes to a splicing enhancer sequence in exon 51 that causes the splicingosome ignores exon 51 and directly read exon 52 in the frame, thus producing shorter yet semi-functional dystrophin proteins [17]. Since etepiliren is suitable for only 13–14% of DMD patients with specific mutations, golodirsen [122], viltolarsen [123], and casimersen [124] have been approved for the treatment of DMD with particular splicing defects. These drugs induce exon 53 or 45 skippings, thereby promoting the expression of dystrophin proteins. Together with etepiliren, these four drugs are third-generation ASO drugs belonging to PMOs sophisticated chemical modifications. Because of the urgency of the patient’s clinical situation and ASO medications may be modified in a sequence-specific manner, patient-customized oligonucleotide treatment has been developed. Milasen is the first patient-customized ASO for neuronal ceroid lipofuscinosis 7 disease (a fatal neurodegenerative disease). It showed an acceptable side-effect during the therapy [125].

Several clinical trials are being conducted to test the efficacy of ribozymes in the treatment of solid tumors, HIV and other diseases [126]. RPI-4610 (Angiomyzome) is a ribozyme that inhibits angiogenesis by targeting the vascular endothelial growth factor receptor one (VEGFR1) mRNA. However, the poor efficacy prevents its clinical development [127]. OZ1 is a tat-vp5-specific anti-HIV ribozyme that increases the number of CD4+ lymphocytes when administered to autologous CD4+ hematopoietic progenitor cells. This suggests that cell-mediated delivery of genes is a safe therapeutic approach for HIV patients and is likely to be a conventional treatment for HIV [128]. Although the results obtained in the initial clinical trials were positive, further investigation is required to determine the stability, in vivo activity, tissue-specific delivery, and long-term expression of ribozymes [126].

**RNAi-based therapy**

*Patisiran* and *GalNAc-conjugated siRNAs*. Three siRNA drugs (patisiran, givosiran, and lumasiran) have been approved by FDA to date, while seven siRNA candidates (inclisiran, vutrisiran, fitusiran, cosdosiran, nedosiran, tivansiran, and teprasiran) are undergoing Phase III clinical trials.

Patisiran is the first FDA-approved RNAi-based drug for treating hTTR with polyneuropathy, ushering in a booming new era for RNAi therapeutics [129]. Similar to inotersen [130], the patisiran siRNA (ALN-18328) silences all potential mRNAs with coding region mutations by targeting the 3′ UTR of the TTR gene [129]. Aplyn above developed the stabilization chemistry-GalNAc delivery platform to improve the clinical efficacy of siRNA medications. To date, approximately one-third of RNAi drugs in clinical trials are GalNAc-conjugated siRNAs. Revusiran was the first GalNAc–siRNA drug that significantly increased asialoglycoprotein receptors uptake for hepatic delivery [131]. Unfortunately, it was discontinued due to an imbalance in fatalities in the “ENDEAVOUR” phase III clinical trial (NCT02319005) [132]. Despite the failure of revusiran, Aplyn above has continued to develop GalNAc–siRNA conjugates for therapeutic usage by strategically positioning chemical modifications within the siRNA that can impart extra stabilization against nucleases activity [133]. Givosiran [134] and Lumasiran [135], the second and third siRNA drugs approved by the FDA, have demonstrated that these GalNAc-conjugated, subcutaneously delivered siRNAs are well tolerated, significantly decrease the target mRNA levels, and have a low hazard profile. Other prominent companies have also utilized fully chemically modified, metabolically stabilized RNAi with varying secondary structures and chemical modification patterns. The RNAi therapy extends beyond the liver to other organs and targets various diseases ranging from rare to common diseases affecting larger patient populations. Quark Pharmaceuticals, for instance, has developed drugs to treat kidney injury (QPI-1002) and eye diseases (QPI-1007). Currently, the pharmaceutical industry’s focus has shifted swiftly toward RNAi drugs for cancer treatment. SiG12D LODER (Local Drug EluteR) is a biodegradable polymeric matrix that contains KRASG12D siRNA (siG12D) drug for the treatment of pancreatic ductal adenocarcinoma (NCT01188785) [136]. Additionally, TKM-080301 (Plk1 inhibitor) has been developed for hepatocellular carcinoma and Atu027 (against protein kinase N3) for advanced solid tumors [137].

*Beyond siRNA*. miRNA inhibitors (Anti-miRs) and miRNA mimics can be used to down- or upregulate miRNAs. Miravirsen (SPC3649) and RG-101 are anti-miRs targeting miR-122 for treating hepatitis C virus infection [138]. The miR-34a mimic, MRX34, is the first...
Table 2. RNA-based drugs with either FDA approval or in late phase 3 clinical trials.

| RNA class | Drug | Alternative name | Sponsor | Indication | Target gene | Target organ | Chemical modifications or delivery method | Administration route | Updated states | Comments | References |
|-----------|------|------------------|---------|------------|-------------|-------------|--------------------------------------------|----------------------|---------------|----------|------------|
| ASO       | Fomiviren | VitraVene, ISIS 2922 | Ionis Pharmaceuticals | Cytomegalovirus, Retinitis, HIV Infections | Immediate early region 2 (IE2) mRNA | Eye | 2'-H | Intravitreal | Completed NCT00002167, NCT00002235 | The first FDA-approved ASO medication (1998), withdrawal in Europe and USA in 2002 |
|           | Mipomersen | Kynammo™, ISIS 301012 | Kastle and IonisTherapeutics | Hypercholesterolemia, Atherosclerosis, Coronary Artery Disease | Apolipoprotein B (ApoB) mRNA | Liver | 2'-MOE | Subcutaneous | Completed NCT01599948, NCT00607373, NCT01475825, NCT00770146, NCT00694109, NCT00794664, NCT00706949 | The second-generation of ASOs ("Gapmers" ASO) |
|           | Nusinersen | ISIS 420915, SPINRAZA | Ionis Pharmaceuticals | Hereditary ATTR | Hepatic transthyretin mRNA | Liver | 2'-MOE | Subcutaneous | Completed NCT01737398 | |
|           | Nusinersen | ISIS 396443, SPRINRAZA | Ionis Pharmaceuticals | Hereditary ATTR | Survival of motor neuron 2 (SMN2) pre-mRNA splicing (exon 7 inclusion) | Central nervous system | 2'-MOE, Fully modified | Intrathecal | Completed NCT02292537 | Approved in 2016 |
|           | Nusinersen | ISIS 396443, SPRINRAZA | Ionis Pharmaceuticals | Spinal Muscular Atrophy (SMA) | Survival of motor neuron 2 (SMN2) pre-mRNA splicing (exon 7 inclusion) | Muscle | 2'-MOE, PMO | Intravenous | Completed NCT02295522 | The third-generation of ASO medications with advanced chemical modifications |
|           | Eteplirsen | AVI-4658, EXONDYS 51™ | Sarepta Therapeutics | Duchenne Muscular Dystrophy (DMD) | DMD pre-mRNA splicing (exon 51 skipping) | Muscle | 2'-MOE, PMO | Intravenous | Completed NCT02250381 | |
|           | Golodirsen | SBP-4053, VONYDIS 53™ | Sarepta Therapeutics | Duchenne Muscular Dystrophy (DMD) | DMD pre-mRNA splicing (exon 51 skipping) | Muscle | 2'-MOE, PMO | Intravenous | Recruiting NCT02500381 | |
|           | Viltolarsen | VITOPROSA, NS-065, NCP-01 | NS Pharma | Duchenne Muscular Dystrophy (DMD) | DMD pre-mRNA splicing (exon 53 skipping) | Muscle | 2'-MOE, PMO | Intravenous | Recruiting NCT04768062, NCT04687020, NCT04060199 | |
|           | Casimersen | SRP-4045, AMONDYS 45™ | Sarepta Therapeutics, Inc. | Duchenne Muscular Dystrophy (DMD) | DMD pre-mRNA splicing (exon 45 skipping) | Muscle | PMO | Intravenous | Recruiting NCT03532542, NCT02500381 | |
| RNAi      | Patisiran | ALN-TTR02, ONPATTRO™ | Alnylam Pharmaceuticals | hATR | TTR mRNA | Liver | PS, 2'-O-Me, 2'-F (LNP) | Intravenous | NCT03862807, NCT01960348 | The first siRNA drug approved by FDA (2018) |
|           | Givosiran | ALN-A51, GW288 | Alnylam Pharmaceuticals | Acute Hepatic Porphyrina | Aminolevulinate synthase 1 (ALAS1) mRNA | Liver | PS, 2'-O-Me, 2'- F GalNAc | Subcutaneous | NCT03386816 | The second siRNA drug approved by FDA (2019) |
|           | Lumasiran | ALN-GO1, OKLUMO | Alnylam Pharmaceuticals | Primary Hyperoxaluria Type 1 (PH1) | Hydroxypyruvate oxidase 1 (HPO1) mRNA | Liver | PS, 2'-O-Me, 2'-F GalNAc | Subcutaneous | NCT03905694, NCT03681184, NCT04152200 | The third siRNA drug approved by FDA (2020) |
|           | Inclisiran | ALN-PCSSC, LEQMO | Alnylam and Novartis Pharmaceuticals | Hypercholesterolemia, Atherosclerotic Cardiovascular disease, Renal impairment | Proprotein convertase subtilisin 3 (PCSK9) mRNA | Liver | PS, 2'-O-Me, 2'- F GalNAc | Subcutaneous | NCT03397121 | Expand siRNA clinical portfolio beyond just orphan diseases, Provide sustained reductions in low-density lipoprotein (LDL) cholesterol levels with infrequent dosing (every 6 months). |
|           | Vitravene | ALN-TTRSC02 | Alnylam Pharmaceuticals | hATR | TTR mRNA | Liver | PS, 2'-O-Me, 2'- F GalNAc | Subcutaneous | May prove to be a more clinically utilizes, effective treatment | NCT04133149, NCT03759379 |
| RNA class | Drug | Alternative name | Sponsor | Indication | Target gene | Target organ | Chemical modifications or delivery method | Administration route | Updated states | Comments | References |
|-----------|------|-----------------|---------|------------|-------------|--------------|---------------------------------|---------------------|---------------|----------|-----------|
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
| Fitusiran  | ALN-AT3SC | Alnylam Pharmaceuticals and Sanofi Genzyme | Hemophilia A / B | Antithrombin mRNA | Blood | PS, 2'-O-Me, 2'-F GalNAc | Subcutaneous | Phase III completed; NCT03974113 NCT03417702 |          |          |          |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
| Nedosiran  | DCR-PHXC | Dicerna Pharmaceuticals | Primary Hyperoxaluria | Hepatic lactate dehydrogenase mRNA | Liver | GalNAc | Subcutaneous | Phase III enrolling by invitation; NCT04043402 |          |          |          |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
| Teprarinon | QPI-1002 | Quark Pharmaceuticals | Cardiac surgery | p53 mRNA | Kidney | 2'-O-Me | Intravenous | Phase III completed; NCT02610296 | Compare with Lumasiran, it will have a potentially wider scope, since it is not limited to just PH1 patients. |          |          |           |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
| QPI-1007   | Quark Pharmaceuticals | Primary angle-closure glaucoma | Caspase 2 mRNA | Eye | 2'-O-Me | Intravitreal | Phase II/III terminated; NCT02341560 |          |          |          |
| Tivanisiran | SYL-1001 | Sylentis, S.A. | Dry eye disease | Transient receptor potential cation channel subfamily V member 1 (TRPV1) mRNA | Eye | Unmodified | Topical eye drop | Phase III completed; NCT03106664 |          |          |           |
|            |      |                 |         |            |             |              |                                 |                     |               |          |           |
| Aptamer    | Pegaptanib | Macugen® | Diabetic Macular Edema | VEGF (165 isoform) | Eye | Pegylated, all PQ, 2'-F, and 2' -OMe G and A methylated | Intravitreal injection | Phase IV completed; NCT01486238 NCT01486238 NCT00406107 NCT00324116 | [146] |          |           |
| mRNA vaccine | BNT162b2 | Comirnaty® | Coronavirus disease 2019 (COVID-19) | Encodes the SARS-CoV-2 spike protein | Immune system | Nucleoside-modified, lipid nanoparticle-formulated | Interventional | Completed NCT04816669 NCT04897944 NCT04713553 NCT05030974 | The first two FDA-approved SARS-CoV-2 vaccines with >94% effectiveness in phase III clinical trial | [147] |          |           |
| mRNA-1273  | Moderna Therapeutics | COVID-19 | Encodes the SARS-CoV-2 spike protein | Immune system | Lipid nanoparticle-formulated | Interventional | Completed NCT05030974 |          |          |          | [148] |
cancer-targeted miRNA drug [139]. However, none of them are currently in clinical usage

MTL-CEBPA is the first saRNA in clinical trials. It regulates hepatic and myeloid functions, as well as numerous oncogenic processes by upregulating the transcription factor CCAAT/enhancer-binding protein alpha (C/EBP-α) [140, 141]. The encouraging results promote the establishment of a clinical trial combining MTL-CEBPA with an anti-PD-1 checkpoint inhibitor or radiofrequency ablation to treat solid tumors [142].

CRISPR/Cas-based genome therapy
The first human clinical trial on applying CRISPR/Cas gene editing involved using ex vivo Cas9 to knockout PD-1 in autologous T cells (NCT03399448) [143]. In addition, β-thalassemia was the first human trial applying CRISPR/Cas to genetic diseases (NCT03655678). The first clinical trial using CRISPR/Cas genome editing to treat retinal defects was EDIT-101 (NCT03872479) [144]. These CRISPR/Cas-based clinical trials have provided a foundation for further genome-editing clinical trials on zinc-finger nucleases [145].

Aptamer
Pegaptanib (Macugen) is the first FDA-approval aptamer drug targeting VEGF to treat age-related macular degeneration [146]. Numerous other aptamers are in the preclinical or clinical development pipeline for possible treatment of diseases, such as visual disorders, coagulation, oncology and inflammation [54].

mRNA vaccine
In the last few decades, mRNA vaccines have gained widespread applications. In 2020, the outbreak of coronavirus disease 2019 (COVID-19) stimulated the most rapid development of mRNA vaccines in history.

Vaccines targeting infectious diseases represent the most advanced application of mRNA therapies. So far, mRNA vaccines for various infectious diseases, including influenza, Zika, and respiratory syncytial virus, have been developed [57]. During the ongoing COVID-19 pandemic, mRNA-based vaccines have proven effective against the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The Pfizer–BioNTech vaccine BNT162b2 (Comirnaty) [147] and the Moderna vaccine mRNA-1273 [148] were the first two FDA-approved SARS-CoV-2 vaccines with >94% effectiveness in phase III clinical trial. Both vaccines employ LNPs formulated using ionizable lipid and a nucleoside-modified mRNA. All uridines in the mRNA are substituted with N1-methylpseudouridine to improve mRNA translation. The mRNA sequence encodes the SARS-CoV-2 spike protein with two proline alterations that give the protein a prefusion shape [149].

The application of mRNA-based cancer vaccines has been recently reviewed [58, 73, 150, 151]. To date, over 20 mRNA-based vaccines have undergone clinical trials as potential preventive strategies for solid tumors, such as melanoma, non-small cell lung cancer, and colorectal carcinoma. In most clinical trials, mRNA cancer vaccines are co-administered with checkpoint modulators (PD-1, CTLA-4, and TIM3) or cytokine cocktails to enhance antitumor efficacy.

More recent advances for RNA-based therapies
Apart from miRNA antagonist and miRNA mimic, artificial circular RNA (circRNA) sponges (circmirs) are promising therapeutic miRNA antagonists. circRNAs are a subclass of ncRNAs that exist as continuous loop RNAs due to the lack of free 3′ and 5′ ends. They are resistant to nuclease degradation and more stable than linear RNAs. The most frequently described function of circRNAs is acting as miRNA sponges. These circRNAs contain multiple miRNA-binding sites for miRNAs binding, preventing the interaction between miRNA and their canonical mRNA target gene [152, 153]. Recent findings reveal that engineered circmirs are efficient sponges of miR-132 and –212 to attenuate pressure overload-induced hypertrophy in vivo in a mouse model. These circmirs also show greater in vitro efficacy than the current gold standard antagoniRs in inhibiting miRNA function [154]. Besides, circRNAs with minimized immunogenicity are potent protein kinase R inhibitors, which efficiently suppress protein kinase R activation 105- to 106-fold higher than reported chemical compounds (C16 and 2-AP) [155]. Moreover, circRNA vaccines against SARS-CoV-2 and emerging variants elicit a higher and more durable immune response [156]. In fact, increasing research has focused on noncoding RNA therapeutics, which have been widely reviewed [157–159].

CONCLUSION AND FUTURE PERSPECTIVES
Various RNA-based approaches have been applied to experiments and clinical trials. Commoditized ASOs, siRNAs, antagonirs and aptamers are widely used for cell and animal experiments. Several ASOs, siRNAs, aptamer and mRNA vaccines have been approved for clinical application. These approaches allow for the down- or up-regulation of specific mRNA expression and inhibition of ncRNA functions by targeting particular RNA sequences. However, the most significant obstacle preventing the widespread usage of RNA-based approaches is the difficulty of efficiently delivering such drugs to target organs and tissues apart from the liver. In addition, off-target binding [162], sequence-induced toxicity, and oversaturation of the endogenous RNA processing pathway [163] affect the effectiveness of RNA-based approaches.

Chemical modification is one of the most promising strategies for delivering RNA-based drugs. Modification of the nucleic acid backbone, ribose ring, and nucleobase itself has been widely used to optimize drug-like characteristics for enhanced delivery. For example, extensive chemical modifications allow the delivery of gaperm ASOs to various tissues without an extra delivery agent. To date, eight of the ten approved oligonucleotide treatments are applied without an additional delivery vehicle. However, some unnatural nucleotides may be harmful. For instance, LNA-modified nucleic acids were found to cause severe hepatotoxicity [164]. Therefore, bioengineered RNAi agents (BERAs), a newly-developed in vivo RNA agent carrying no or minimal post-transcriptional modifications, have shown good application prospects [165].

Developing nanocarriers for RNA drug delivery provides excellent hepatic transport. Non-liver systemically administered nanomedicines require further investigation. Advances in imaging techniques (e.g., electron microscopy, super-resolution fluorescence microscopy, single-particle tracking 3, etc.) [166] and omics-based approaches [167] have allowed scientists to investigate intracellular delivery processes, thereby boosting the innovation of nanoformulations and rational design of advanced delivery vehicles.

Apart from the sequence-based method, small-molecule-based therapy to target RNA would be preferred in many cases [168]. Evrydsli (risdiplam) is the first FDA-approved orally bioavailable small-molecule inhibitor targeting SMN2 pre-messenger RNA splicing. It is
synthesized efficiently and can be easily administered [169]. Tutorials about generating RNA-targeted small molecules have been discussed in other reviews [5, 170].

In summary, combining RNA chemical modifications and conjugation with nanocarrier systems can improve the efficiency of RNA drug delivery. Further research on RNA-based therapeutics, including RNA molecules as therapeutic drugs and targeting RNA with small molecules, will lead to more RNA-based therapeutics for patient treatment.

DATA AVAILABILITY
All relevant data are included in this paper.

REFERENCES

1. Zamecnik PC, Stephenson ML. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc Natl Acad Sci USA. 1978;75:280–4.
2. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001;411:494–8.
3. Wang F, Zurose K, Watts JR. RNA therapeutics on the rise. Nat Rev Drug Discov 2020;19:441–2.
4. Crooke ST, Witzum JL, Bennett CF, Baker BF. RNA-targeted therapeutics. Cell Metab. 2018;27:714–39.
5. Falese J, Donlic A, Hargrove A. Targeting RNA with small molecules: from fundamental principles towards the clinic. Chem Soc Rev 2021;50:2224–43.
6. Dixon SJ, Stockwell BR. Identifying druggable disease-modifying gene products. Curr Opin Chem Biol. 2009;13:549–55.
7. Sahin U, Karikó K, Türeci O. mRNA-based therapeutics—developing a new class of drugs. Nat Rev Drug Discov. 2014;13:759–80.
8. Kaufmann KB, Bünning H, Galy A, Schambach A, Grez M. Gene therapy on the move. EMBO Mol Med 2013;5:1642–61.
9. Wang F, Wang L, Zou X, Duan S, Li Z, Deng Z, et al. Advances in CRISPR-Cas systems for RNA targeting, tracking and editing. Biotechnol Adv. 2019;37:708–20.
10. Adachi T, Nakamura Y. Aptamers: a review of their chemical properties and applications. Biotechnol Adv. 2019;37:708–20.
11. Sullivan SM. Development of ribozymes for gene therapy. J Invest Dermatol. 1994;103 5 Suppl:85s–9.
12. Desterro J, Bak-Gordon P, Carmo-Fonseca M. Targeting mRNA processing as an anticancer strategy. Nat Rev Drug Discov. 2019;18:112–22.
13. Li D, Mastroglia FL, Fletcher S, Wilton SD. Precision medicine through antisense oligonucleotide-mediated exon skipping. Trends Pharm Sci. 2018;39:982–4.
14. Sullivan SM. Development of ribozymes for gene therapy. J Invest Dermatol. 1994;103 5 Suppl:85s–9s.
15. Li D, Mastaglia FL, Fletcher S, Wilton SD. Precision medicine through antisense oligonucleotide-mediated exon skipping. Trends Pharm Sci. 2018;39:982–4.
16. Li D, Mastaglia FL, Fletcher S, Wilton SD. Precision medicine through antisense oligonucleotide-mediated exon skipping. Trends Pharm Sci. 2018;39:982–4.
17. Li D, Mastaglia FL, Fletcher S, Wilton SD. Precision medicine through antisense oligonucleotide-mediated exon skipping. Trends Pharm Sci. 2018;39:982–4.
18. Li D, Mastaglia FL, Fletcher S, Wilton SD. Precision medicine through antisense oligonucleotide-mediated exon skipping. Trends Pharm Sci. 2018;39:982–4.
19. Li D, Mastaglia FL, Fletcher S, Wilton SD. Precision medicine through antisense oligonucleotide-mediated exon skipping. Trends Pharm Sci. 2018;39:982–4.
20. Melton DA. Injected anti-sense RNAs specifically block messenger RNA translation in vivo. Proc Natl Acad Sci USA. 1985;82:144–8.
21. Baker BF, Lot SS, Krinzel J, Cheng-Flourny S, Villett P, Sasmor HM, et al. Oligonucleotide-europium: a complex conjugate designed to cleave the 5' cap structure of the ICAM-1 transcript potentiates antisense activity in cells. Nucleic Acids Res. 1999;27:1547–51.
22. Vickers TA, Wyatt JR, Burckin T, Bennett CF, Freier SM. Fully modified 2’MOE oligonucleosides redirect polyadenylation. Nucleic Acids Res. 2001;29:1293–9.
23. Liang XH, Shen W, Sun H, Migawa MT, Vickers TA, Crooke ST. Translation efficiency of miRNAs is increased by antisense oligonucleotides targeting upstream open reading frames. Nat Biotechnol. 2016;34:875–80.
24. Liang XH, Sun H, Shen W, Wang S, Yao J, Migawa MT, et al. Antisense oligonucleotides targeting translation inhibitory elements in 5’ UTRs can selectively increase protein levels. Nucleic Acids Res. 2017;45:9528–46.
25. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov. 2014;13:622–38.
115. Seller S, Kocabay S, Nekola K, Krombach F, Liedl T, Rehberg M. DNA nanotubes as intracellular delivery vehicles in vivo. Biomaterials. 2015;53:453–63.

116. Mohri K, Nishikawa M, Takahashi N, Shiomi T, Matsuoka N, Ogawa K, et al. Design and development of nanosized DNA assemblies in polypod-like structures as efficient vehicles for immunostimulatory CpG motifs to immune cells. ACS Nano. 2012;6:5931–40.

117. Pinheiro AV, Han D, Shih WM, Yan H. Challenges and opportunities for structural DNA nanotechnology. Nat Nanotechnol. 2011;6:763–72.

118. Perry CM, Balfour JA, Fomvirsens. Drugs. 1999;57:375–80.

119. Anderson KP, Fox MC, Brown-Drenger V, Martin MJ, Azad RF. Inhibition of human cytomegalovirus immediate-early gene expression by an antisense oligonucleotide complementary to immediate-early RNA. Antimicrob Agents Chemother. 1996;40:2004–11.

120. Blom DJ, Raal FJ, Santos RD, Marais AD. Lomipatide and mipomersen-inhibiting microsomal triglyceride transfer protein (MTP) and apoB100 synthesis. Curr Atheroscler Rep. 2019;21:48.

121. Aartsma-Rus A. FDA approval of nusinersen for spinal muscular atrophy makes 2015;23:1380–86.

122. Heo YL. Golodispers: first approval. Drugs 2020;80:329–33.

123. Dhillon S, Viltolarsen: first approval. Drugs 2020;80:1027–31.

124. Shirley M. Casimersen: first approval. Drugs 2019;52:1528–92.

125. Kim J, Hu C, Moufawad El Achkar C, Black LE, Douville J, Larson A, et al. Patient-validated oligonucleotide therapy with advanced liver cancer: a first-in-human, multicenter, open-label, phase I trial. Clin Cancer Res. 2020;26:3936–47.

126. Morrow PK, Murthy RK, Ensor JD, Gordon GS, Margolin KA, Elias AD, et al. Clinical Scale Zinc Finger nuclease-mediated gene editing of PD-1 in tumor-infiltrating lymphocytes for the treatment of metastatic melanoma. Mol Ther. 2017;25:715–21.

127. Scott LJ. Givosiran: clinical proof of concept for a novel hepatocyte-targeting GalNAc-siRNA conjugate. Mol Ther. 2017;25:71–8.

128. Barber K. Alynlam terminates reusavirus program, stock plunges. Nat Biotechnol. 2016;34:1213–4.

129. Nair JK, Attarwala H, Sehgal A, Wang Q, Aluri K, Zhang X, et al. Impact of enhanced metabolic stability on pharmacokinetics and pharmacodynamics of GalNAc-siRNA conjugates. Nucleic Acids Res. 2017;45:10969–73.

130. Scott LJ. Givosiran: first approval. Drugs 2020;80:335–9.

131. Aartsma-Rus A. FDA approval of nusinersen for spinal muscular atrophy makes 2015;23:1380–86.

132. Heo YL. Golodispers: first approval. Drugs 2020;80:329–33.

133. Shirley M. Casimersen: first approval. Drugs 2019;52:1528–92.

134. Corbett KS, Edwards DK, Leist SR, Albina OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 2020;5856:567–71.

135. Heine A, Juranek S, Brossart P. Clinical and immunological effects of mRNA vaccines in malignant diseases. Mol Cancer. 2021;20:52.

136. Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. Mol Cancer. 2021;20:41.

137. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495:333–8.

138. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495:384–8.

139. Devlin JM, Culver EL, Weisberg E, Smith SR, Li Y. RNA interference–a potential tumor suppressor and therapeutic target. Genes Cancer. 2010;1:183–94.

140. Sarker D, Plummer R, Meyer T, Sodergren MH, Basu B, Chee CE, et al. MTL-CEBPA, a small activating RNA therapeutic upregulating C/EBPα, in patients with hepatocellular carcinoma. Curr Pharm Biotechnol. 2017;18:677–9.

141. Setten RL, Lightfoot HL, Habib NA, Rossi JJ. Development of MTL-CEBPA: small molecules against RNA targets attract big backers. Nat Rev Drug Discov. 2017;16:167–79.

142. He B, Peng W, Huang J, Zhang H, Zhou Y, Yang X, et al. Modulation of metabolic functions through Cas13d-mediated gene knockdown in liver. Protein Cell. 2020;11:518–24.

143. Chou C, Hu X, Tang C, Liu W, Wang S, Zhou Y, et al. CasRx-mediated RNA targeting prevents choroidal neovascularization in a mouse model of age-related macular degeneration. Nat Sci Rev. 2020;7:835–7.

144. Grimm D, Streetz KL, Jopling CL, Storm TA, Pandey K, Davis CR, et al. Fatality in mice due to overexpression of cellular microRNA/shorn hairpin RNA pathways. Nature. 2006;441:537–41.

145. Sarker D, Plummer R, Meyer T, Sodergren MH, Basu B, Chee CE, et al. MTL-CEBPA, a small activating RNA therapeutic upregulating C/EBPα, in patients with advanced liver cancer: a first-in-human, multicenter, open-label, phase I trial. Clin Cancer Res. 2020;26:3936–46.

146. Setten RL, Lightfoot HL, Habib NA, Rossi JJ. Development of MTL-CEBPA: small molecules against RNA targets attract big backers. Nat Rev Drug Discov. 2017;16:813–5.

147. Warner KD, Hajdin CE, Weeks KM. Principles for targeting RNA with drug-like small molecules. Nat Rev Drug Discov. 2018;17:547–58.

**AUTHOR CONTRIBUTIONS**

HJ, XM, and YZ conceptualized the paper; YZ performed the literature collection and wrote the paper; LZ and HU edited and revised the paper. All authors read and approved the final paper.

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COMPETING INTERESTS
The authors declare no competing interests.

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