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RNA therapeutics: updates and future potential

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Recent advancements in the production, modification, and cellular delivery of RNA molecules facilitated the expansion of RNA-based therapeutics. The increasing understanding of RNA biology initiated a corresponding growth in RNA therapeutics. In this review, the general concepts of five classes of RNA-based therapeutics, including RNA interference-based therapies, antisense oligonucleotides, small activating RNA therapies, circular RNA therapies, and messenger RNA-based therapeutics, will be discussed. Moreover, we also provide an overview of RNA-based therapeutics that have already received regulatory approval or are currently being evaluated in clinical trials, along with challenges faced by these technologies. RNA-based drugs demonstrated positive clinical trial results and have the ability to address previously "undruggable" targets, which delivers great promise as a disruptive therapeutic technology to fulfill its full clinical potentiality.

RNA, RNA therapeutics, mRNA vaccine, RNAi

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Introduction

From the discovery of messenger RNA (mRNA) in 1961 (Brenner et al., 1961; Gros et al., 1961) to the first mRNA vaccine approved for marketing in 2021, 60 years have elapsed. During this 60-year period, the field of RNA has developed dramatically. In 1977, Roberts and Sharp discovered RNA splicing and were awarded the 1993 Nobel Prize in Physiology or Medicine (Chow et al., 1977). In 1982, Cech discovered the self-splicing ribozyme of Tetrahymena RNA and shared the 1989 Nobel Prize in Chemistry with Altman for their discovery (Kruger et al., 1982). This discovery has turned a new page in the RNA field, and the RNA molecule was recognized as a biomolecule with dual replication and catalytic functions. In 1993, Ambros discovered the first microRNA (miRNA) (lin-4) (Lee et al., 1993); in 1998, Fire and Mello discovered the RNA interference (RNAi) phenomenon and received the 2006 Nobel Prize in Physiology or Medicine (Fire et al., 1998). RNAi became an important tool for gene function research, and 20 years later, the first RNAi drug was successfully approved for marketing. In 1999, Ramakrishnan, Steitz, and Yonath completed the ribosome crystal structure to demonstrate the ribosome as a ribozyme and were awarded the 2009 Nobel Prize in Chemistry (Ban et al., 2000; Schluenzen et al., 2000; Wimberly et al., 2000). In 2011, the discovery of CRISPR gene-editing technology by Charpentier and Doudna yielded the 2020 Nobel Prize in Chemistry and revealed sgRNA-guided DNA cleavage as a critical gene therapeutic tool (Jinek et al., 2012). The brief 60-year history of RNA molecules yielded five Nobel prizes for the scientific discoveries related to RNA, which exemplifies its significance in the chemical and biological fields and underlines its versatility as an indepensible technology that will revolutionize

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medicine.

RNA-based drugs are mainly divided into two major classes: (i) oligonucleotide drugs synthesized by chemical methods, such as antisense oligonucleotides (ASOs), siRNAs (small-interfering RNAs), and RNA aptamers; (ii) macromolecular RNA drugs synthesized via *in vitro* transcription, like mRNA drugs. Nearly 108 oligonucleotide drugs are entering the clinical pipeline worldwide, including ASOs, siRNAs, aptamers, single guide RNAs (sgRNAs), and miRNAs. Additionally, 56 mRNA drugs worldwide are also entering the clinical pipeline. As of the date of this paper, a total of 16 nucleic acid drugs have been approved for marketing worldwide, as seen in Table 1. With the launch of Nusinersen, developed by Biogen and Ionis, the world's first oligonucleotide drug treating spinal muscular atrophy (SMA) emerged and is currently the highest-selling oligonucleotide drug.

Due to the large molecular structure and negative charge, oligonucleotide drugs are easily degraded by RNases and are difficult to penetrate cell membranes, and have fallen into a trough from 2008 to 2013 due to safety issues and delivery system problems. Drug delivery plays a crucial role in protecting RNA structure, increasing targeting capacity, lowering the dose administered, and reducing toxic side effects. With the technological breakthrough of key delivery systems, the oligonucleotide drug industry has ushered in vigorous development. RNAi therapy is an essential strategy for treating genetic diseases and "undruggable" targets.

siRNA and miRNA are meaningful gene silencing tools; four siRNA drug candidates have been approved, however many miRNA drugs have mostly terminated due to safety issues, with only five candidates continuing into clinical development, and none has entered the Phase 3 clinical trial. The most significant difference between siRNA and miRNA drugs is their mechanism of action. siRNAs are perfect complementary targeting gene silencing sequence and enter the body to act on specific target gene. In contrast, a miRNA can regulate hundreds or thousands of genes, and one gene can be regulated by many different miRNAs. Therefore, it is difficult to designate miRNA to regulate a specific gene and can result in unexpected side effects. Only by addressing the specificity issue of miRNA drugs, miRNA can be applied in the clinical setting.

As a technology platform with great potential, mRNA-based therapies can be used for infectious disease prevention, tumor immunotherapy, protein replacement therapy, and even gene editing. The therapeutic avenues of mRNA can be categorized into three classes: prophylactic vaccine, therapeutic vaccine, and therapeutic drugs. Comirnaty, developed by Pfizer and BioNTech, and Spikevax, developed by Moderna, were granted the United States Food and Drug Administration (FDA) emergency use authorizations in December 2020 to prevent COVID-19 caused by SARS-CoV-2. In 2021, Comirnaty and Spikevax's sales reached $36.8 billion and $17.7 billion, respectively. The two vaccines shined in the prevention of the global pandemic, heralding the official entry of mRNA technology into the era of commercialization.

RNA molecules have long been recognized as playing essential roles in cellular processes spanning from gene regulation and expression to enzymatic reactions (Xue et al., 2020). RNA therapeutics is a rapidly expanding category of drugs and therapies that have experienced unprecedented speed from benchside to clinical practice. It was originally

Table 1 Globally listed RNA-based drugs

Class	Generic name	Indication	Target	Modification/Delivery	Year of approval
ASO	Fomivirsen	Cytomegalovirus retinitis	CMV UL123	Thiophosphate oligo	1998 (discontinued)
	Mipomersen	HoFH	AP08	Thiophsphate/2′-MOE	2013 (discontinued)
	Nusinersen	Spinal muscular atrophy	Exon 7 of SMN2	Thiophsphate /2′-MOE	2016
	Eteplirsen	Duchenne muscular dystrophy	Exon 51 of DMD	PMO	2016
	Inotersen	Familial amyloid polyneuropathy	TTR	Thiophsphate /2′-MOE	2018
	Volanesorsen	Familial chylomicronemia syndrome	ApoC3	Thiophsphate /2′-MOE	2019
	Golodirsen	Duchenne muscular dystrophy	Exon 53 of DMD	PMO	2019
	Viltolarsen	Duchenne muscular dystrophy	Exon 53 of DMD	PMO	2020
	Casimersen	Duchenne muscular dystrophy	Exon 45 of DMD	PMO	2021
siRNA	Patisiran	Familial amyloid polyneuropathy	TTR	LNP	2018
	Givosiran	Acute Hepatic porphyria	ALAS1	GalNAc	2019
	Lumasiran	Primary hyperoxaluria type 1	HAO 1	GalNAc	2020
	Inclisiran	Hypercholesterolemia	PCSK9	GalNAc	2020
Aptamer	Pegaptanib	nAMD	VEGF-165	PEG	2004 (discontinued)
mRNA	Tozinameran	SARS-CoV-2	Spike protein	LNP	2020
	Elasomeran	SARS-CoV-2	Spike protein	LNP	2020

thought that RNA molecules would be a poor therapeutic agent due to their instability and relatively short half-life. However, with the advancements in stabilization chemistry and application for its transient characteristic, RNA molecules as therapeutic agents have shown to be clinically valuable. Here we briefly review the five classes of RNA-based therapies: (i) RNAi-based therapies, (ii) ASO therapies, (iii) small activating RNA (saRNA) therapeutics, (iv) circular RNA (circRNA) therapies, and (v) mRNA-based therapies, and its drug candidates that reached later-stage clinical development.

With hundreds of oligonucleotide and mRNA drug candidates in discovery and preclinical development, more than 150 oligonucleotide drugs and mRNA therapeutics are currently in clinical trials. The years 2018 and 2020 were milestones for the RNAi and mRNA fields as RNA-based therapies were approved for marketing. With more RNA therapeutics under development, the future is bright for RNA-based therapies.

RNAi-based therapies

In recent decades, the discovery of small noncoding RNAs and their role in gene regulation transformed the field of RNA biology. RNAi was first described by Andrew Fire and Craig Mello, who were awarded the Nobel Prize in Physiology or Medicine for their contribution towards RNAi. They discovered the gene silencing mechanism mediated by exogenous RNA in *Caenorhabditis elegans* (Fire et al., 1998). Small noncoding RNA, like siRNA, form complexes known as the RNA-induced silencing complex (RISC), which directs the degradation of a target messenger RNA (mRNA), as depicted in Figure 1. The post-transcriptional gene silencing mechanism of RNAi makes it a powerful tool for controlling gene expression by inhibiting specific base sequences of a gene, revolutionizing drug discovery and development.

Given the specificity and potency of RNAi, many RNAi-based strategies have been developed for therapeutic purposes, including siRNA and miRNA therapeutics, which have shown promising results in clinical trials as medical intervention drugs. By the end of 2021, four RNAi drugs have been approved and authorized by both the European Commission (EC) and the FDA, which yielded proof-of-concept for the efficacy and safety of RNAi therapies. The mechanism of action of siRNA and miRNA therapeutics with examples of their candidate drugs is discussed.

siRNA therapeutics

In 2018, the U.S. FDA and European Medicines Agency (EMA) approved patisiran (ONPATTRO; Alnylam Pharma-

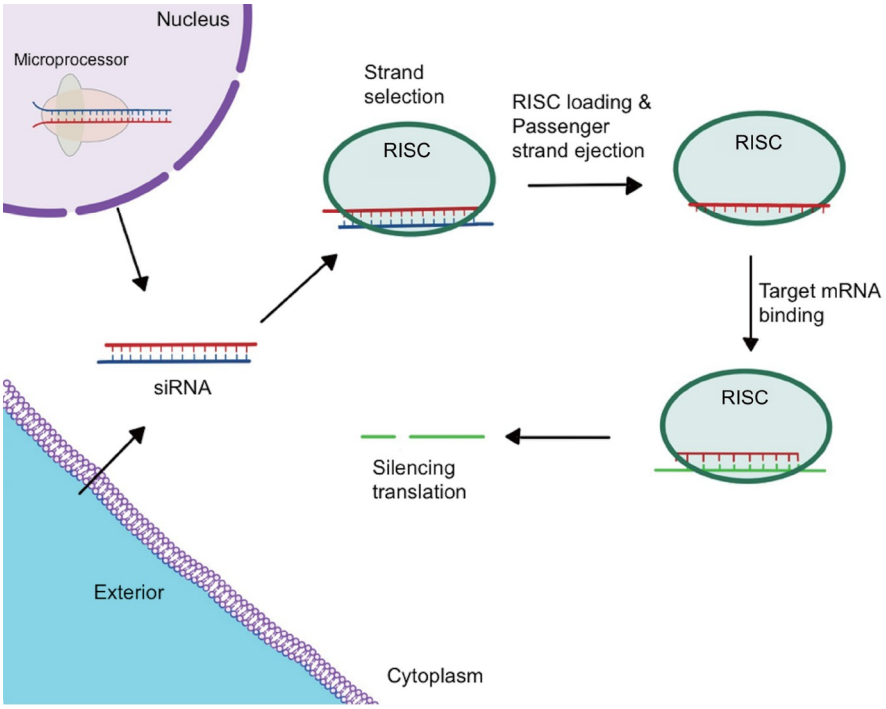

Figure 1 Schematic diagram of the siRNA biogenesis to silence target mRNA. The siRNA precursor is a double-stranded RNA originating endogenously or exogenously by viruses, transposons, or other sources. The precursor is cleaved by enzymes for proper loading of the Argonaute protein and are assisted by a dsRNA-binding protein, which allows for binding of the guide strand to the Argonaute followed by the ejection of the passenger strand. Finally, the silencing activity can occur via target binding and cleavage.

ceuticals), a siRNA drug to treat polyneuropathy caused by hereditary transthyretin (hATTR) amyloidosis in adult patients. This siRNA drug is the first RNAi-based therapy approved, marking a new era for RNAi therapy. Currently, there are four siRNA drugs approved for marketing and many more in clinical trials, as seen in Table 2.

siRNAs belong to the dsRNA class of molecules. As indicated in its name, the length of siRNAs is short at about 18 to 25 nucleotides (nt) in length with or without two overhanging phosphorylated bases at the 3′ ends of each strand (Alshaer et al., 2021; Laganà et al., 2014; Zhang et al., 2021a). The mechanism of siRNA therapeutics harnesses its natural ability to cleave specific sequences of perfectly complementary mRNA, which is carried out by the catalytic domain of Argonaute 2 (Ago2). Synthetic siRNAs designed to mimic Dicer cleavage, known as "Dicer-ready" siRNAs, can be delivered to cells of interest and are directly recognized by and loaded onto the Ago2-RISC complex, making it particularly attractive as a drug (de Fougerolles et al., 2007). By designing a siRNA sequence of desired potency and specificity to the target mRNA, disease-causing mRNA can be silenced (Patrick Walton et al., 2010).

The latest approved siRNA drug for the treatment of ASCVD and HeFH, inclisiran (LEQVIO; Novartis), is a first-in-class *N*-acetylgalactosamine (GalNac)-modified siRNA conjugates that demonstrated a robust decrease in plasma cholesterol levels (Ray et al., 2020). Atherosclerosis is described as a chronic inflammatory disease of the arteries, which results in a chronic build-up of plaques occluding the vessels leading to stenosis and tissue hypoxia and is a major cause of ASCVD (Wolf and Ley, 2019). The pathogenesis of atherosclerosis is complex; however, it is known to be associated with increased low-density lipoprotein cholesterol (LDL-c) levels (Barquera et al., 2015). Inclisiran targets the mRNA of proprotein convertase subtilisin/kexin type 9 (PCSK9); PCSK9 is a serine protease associated with LDL receptor (LDLR) binding, which regulates cholesterol and cytoplasmic apolipoprotein B (ApoB) that is found to increase plasma LDL concentrations (Sun et al., 2018). By inhibiting the production of PCSK9, the reduced PCSK9 mRNA expression decreases LDLR degradation and increases LDL uptake to lower concentrations of LDL-c. Furthermore, PCSK9 inhibition reduces vascular chemokines and autophagy molecules associated with the inflammatory properties of atherosclerosis (Ruotsalainen et al., 2021; Sun et al., 2018).

Phase 2 and 3 clinical trials showed promising results of inclisiran in patients with ASCVD and HeFH (Ray et al., 2017; Hovingh et al., 2020). ORION-9 trial (NCT identifier: NCT033907121) enrolled 482 patients with HeFH; ORION-10 trial (NCT identifier: NCT03399370) was conducted with 1,561 patients with ASCVD; and ORION-11 trial (NCT identifier: NCT03400800) enrolled 1,617 patients with ASCVD or its risk equivalents. In these phase 3 trials, patients received 1.5 mL subcutaneous injections of 284 mg of inclisiran (equivalent to 300 mg of inclisiran sodium) on days 1, 90, 270, and 450 (Ray et al., 2020; Raal et al., 2020). All phase 3 studies reported having met their primary efficacy endpoints. A pooled analysis of the three randomized clinical trials (RCT) mentioned above demonstrated an overall decrease in LDL-c by 51% with two doses a year compared to the placebo group and lowered the major adverse cardiovascular event rate by 24%. Additionally, inclisiran was found to decrease total cholesterol, ApoB, and non-high-density lipoprotein cholesterol levels (Khan et al., 2020). Therefore, inclisiran is effective at LDL-c reduction

Table 2 List of representative siRNA drugs approved for marketing or are currently in/completed phase 2 and phase 3 clinical trials[a)]

Drug Name	Indication	Developer	Status
Patisiran	hATTR amyloidosis	Alnylam	FDA approval in 10/08/2018 (N210922*)
Givosiran	Acute hepatic porphyria (AHP)	Alnylam	FDA approval in 11/20/2019 (N212194*)
Lumasiran	Primary hyperoxaluria Type 1 (PH1)	Alnylam	FDA approval in 11/23/2019 (N214103*)
Inclisiran	Atherosclerotic cardiovascular disease (ASCVD), heterozygous familial hypercholesterolemia (HeFH)	Novartis	FDA approval in 12/22/2021 (N214012*)
Vutrisiran	hATTR amyloidosis	Alnylam	Phase 3 trial (NCT04153149**)
Tivanisiran	Dry eye disease Sjögren syndrome	Sylentis	Phase 3 trial (NCT04819269**)
Fitusiran	Hemophilia A and B	Sanofi Genzyme	Phase 3 trial (NCT03417102**)
Teprasiran	Acute kidney injury	Quark	Phase 3 trial terminated (NCT03510897**)
ARO-APOC3	Hypertriglyceridemia	Arrowhead	Phase 3 trial (NCT05089084**)
Nedosiran	Primary hyperoxaluria type 3 (PH3)	Dicerna	Phase 2 trial (NCT04555486** NCT03847909**)
Olpasiran	Cardiovascular disease	Amgen	Phase 2 trial (NCT04270760**)
Belcesiran	PiZZ alpha-1 antitrypsin deficiency (A1ATD)	Dicerna	Phase 2 trial (NCT04764448**)
ARO-ANG3	Mixed dyslipidemia	Arrowhead	Phase 2 trial (NCT04832971**)

a) *, Application number. **, Clinicaltrials.gov identification number. Data adapted from https://clinicaltrials.gov.

with an infrequent dosing regimen to maintain such effect.

In addition to the efficacy of inclisiran, its safety profile is equally acceptable. The safety profile of the three RCTs was consistent with previous studies and was not associated with abnormal liver function, creatine kinase levels, and platelet count. Injection site adverse events were reported more frequently with inclisiran than placebo (Ray et al., 2020; Khan et al., 2020). Although inclisiran's performance displays a significant decrease in LDL-c and PSCK9 levels, another meta-analysis of the three studies observed no statistically significant difference in myocardial infarction risk in patients with inclisiran compared with placebo (Asbeutah et al., 2020). Overall, inclisiran significantly reduces LDL-c levels by inhibiting hepatic production of PCSK9 with two doses a year while exhibiting an ideal safety profile. The success of inclisiran and its precedent siRNA drugs demonstrate the promising future of siRNA-directed RNAi therapies. This is also the first small nucleic acid drug to treat common chronic diseases.

miRNA therapeutics

miRNA therapeutics made its entry as a novel therapeutic agent for the treatment of varying diseases due to its role in development. As mentioned previously, miRNAs and siRNAs are both small duplex RNA molecules capable of targeting the mRNA through post-transcriptional gene silencing (Lam et al., 2015). However, their biogenesis and mechanism of action differ. The biogenesis of miRNA begins in the nucleus, where its gene transcription occurs and is tightly regulated, as seen in Figure 2. The miRNA duplex–a result of the processed pri-miRNA–associates with the RISC to form the miRISC, guided by the mature miRNA for target recognition. Unlike siRNA, miRNA binds to target mRNAs via partial complementary base pairing and inhibits mRNA translation instead of target mRNA cleavage (Ahmadzada et al., 2018).

There are two approaches for the therapeutics applications of miRNA: miRNA inhibition and miRNA replacement. miRNA inhibition utilizes synthetic single-stranded RNA analogs made complementary to the active strand of the target miRNA and act as miRNA antagonists (also known as anti-miRs or antagomiRs) to inhibit endogenous miRNAs, which is structurally analogous to antisense oligonucleotides (Ahmadzada et al., 2018; Lam et al., 2015). Anti-miRs were first described by Krützfeldt et al. (2005) in which intravenous administration of these chemically modified, cholesterol-conjugated oligonucleotides complementary to the target miRNA reduced miR-16, miR-122, miR-192, and miR-194 levels in mice. The latter approach employs synthetic miRNAs (also known as miRNA mimics) to mimic the function of target miRNAs, prompting mRNA inhibition. miRNA mimics can be used similarly as chemically mod-

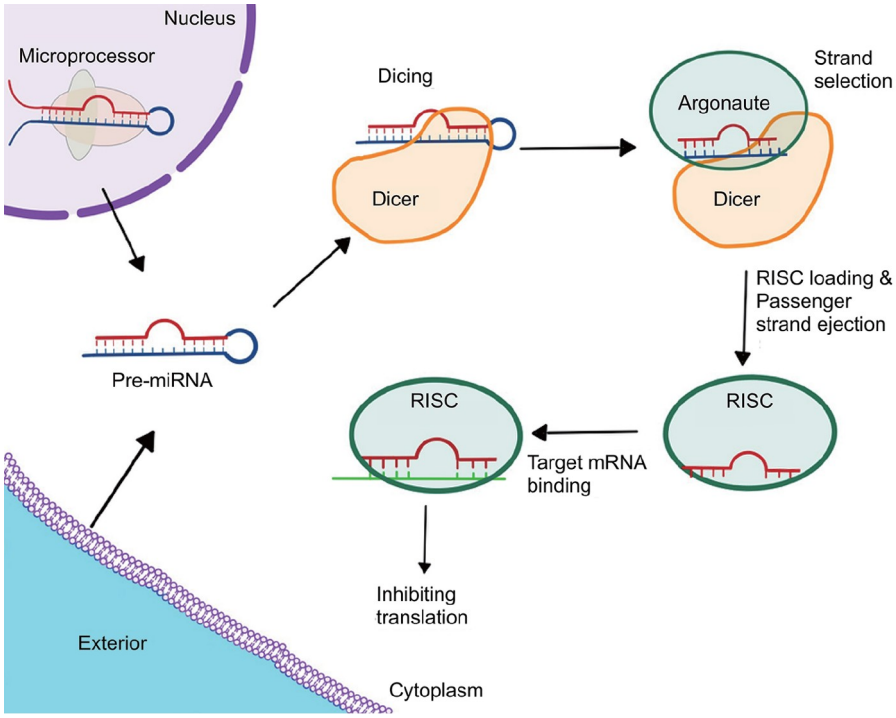

Figure 2 Schematic diagram of miRNA biogenesis and mechanism of action. The precursor miRNA (pre-miRNA) is produced from the primary miRNA (pri-miRNA) consisting of terminal loops and mismatches in the nucleus. Pre-miRNA enters the cytoplasm for enzymatic cleavage of the terminal loop by the Dicer enzyme and generates the miRNA duplex. The following process draws similarities with the biogenesis of siRNA, in which the RISC is formed with its RNA duplex and is loaded onto the argonaute for strand selection. Finally, the mature miRNA is ready for target mRNA binding and degradation.

ified siRNAs with the same guide strand as the target miRNA, in which they are loaded onto the RISC to inhibit the downstream endogenous miRNA. Because of its similarities with typical miRNA, miRNA mimics can potentially contribute as a therapeutic agent for cancer management due to the role of tumor suppressor miRNAs in cancer development. However, there are remaining challenges in the delivery of miRNA mimetic agents (Setten et al., 2019; Otoukesh et al., 2020).

The emergence of miRNA therapeutics has not yet translated into approved drug candidates for medical intervention. To date, there are several miRNA molecules under clinical trials. However, none entered into the phase 3 trials. Currently, lademirsen (RG-012), developed by Regulus and Genzyme (Sanofi), has ongoing patient recruitment for phase 2 trials (NCT identifier: NCT02855268). Lademirsen is an anti-miR drug candidate administered subcutaneously to silence the function of microRNA-21 (miR-21) in patients with Alport syndrome (Hanna et al., 2019). Alport syndrome is an X-linked genetic disorder characterized by chronic kidney disease caused by mutations in the genes encoding for collagen IV. The Gomez et al. (2015) preclinical study found the chemically modified anti-miR-21 oligonucleotides with phosphorothioate linkages and modified sugar moieties enhanced affinity to miR-21 and improved survival for Alport neuropathy mouse models. Clinical data regarding lademirsen have not been released at the time of this review.

In addition to the clinical development of lademirsen, remlarsen (MRG-201), developed by MiRagen Therapeutics (now Viridian Therapeutics), completed phase 2 trials to treat patients with keloid (NCT identifier: NCT03601052). Remlarsen is a microRNA-29 (miR-29) mimic aimed to treat pathological fibrosis. The miR-29 family is found to play a role in fibrosis regulation pathways, in which miR-29 expression levels are lower in fibrotic diseases. Remlarsen mimics miR-29 to repress the formation of fibrosis, such as cutaneous fibrosis (Chakraborty et al., 2021). Gallant-Behm et al. (2019) study summarized the phase 1 clinical data of remlarsen performed in healthy volunteers administered intradermally, which the drug candidate reduced collagen expression and fibroplasia development in incisional skin wounds. Furthermore, the candidate was deemed safe and well-tolerated at all studied doses in the phase 1 study. The phase 1 results suggest a possible effective therapeutic to prevent skin fibrosis; however, the yet to be released phase 2 clinical results conducted in patients with a history of keloids may have more significant clinical implications. Despite the substantial number of preclinical studies in miRNA drugs, only a small number of miRNA drug candidates moved into clinical development, and 50% of miRNA drugs experienced termination or suspension in the clinical development phase (Zhang et al., 2021b). Challenges are faced in developing miRNA drug candidates, such as identifying specific miRNA targets for different diseases and their precise delivery while avoiding degradation when entering cells (Rupaimoole and Slack, 2017).

RNAi-based therapies, like siRNA and miRNA therapeutics, are in a continuously evolving field and hold great therapeutic promise for many diseases. There are remaining challenges that are faced by RNAi drugs, like endosomal escape and delivery to non-liver and non-kidney tissues (Segal and Slack, 2020; Setten et al., 2019). Even though currently only four siRNA therapeutics received regulatory approval, more are expected to follow in the near future. The broad clinical application of gene silencing and safety profile offered by RNAi therapies strengthen its potential.

Antisense oligonucleotides therapies

In 1978, Zamecnik and Stephenson demonstrated *in vitro* viral replication inhibition using a 13-nt long antisense oligonucleotide (ASO) complementary to the target sequence of the Rous sarcoma virus, which opened the potential of ASOs as a form of therapeutics (Zamecnik and Stephenson, 1978). Remarkable advancements in oligonucleotide drug development have been made since this discovery. ASOs are short single-stranded and highly modified nucleic acid analogs designed to target sequence-specific RNA for degradation. There are several mechanisms of action for ASOs, as depicted in Figure 3. The targeted RNAs are usually precursor mRNAs (pre-mRNA) in the nucleus, in which ASOs bind to the polyadenylation recognition site to prevent polyadenylation utilizing nuclear ribonuclease (RNase) H; ASOs can also bind to translation initiation sites of mRNAs in the cytoplasm to inhibit translation (Lieberman, 2018). The chemical modifications of ASOs are critical to its translation as a therapeutic drug for increased stability, specificity, and reduced adverse effects (Damase et al., 2021). Typically, ASOs have a phosphorothioate (PS) linkage between two flanking regions of 2′ modified sugars, forming the backbone. The PS moiety preserves the oligonucleotide from exonucleases and improves stability. This design presents a center PS oligonucleotide gap, giving the name "gapmer", which can activate RNase H1 for target RNA cleavage as the RNA-DNA duplex is formed (Kole et al., 2012; Li et al., 2021). In addition, modifications in phosphorodiamidate morpholino oligomers (PMOs) are also widely used in ASOs, in which the six-membered morpholino ring replaces the five-membered ribofuranose ring with the backbone being linked by phosphorodiamidates instead. This modification also stabilizes the PMO against nucleases but minimizes the reduction of complementary targeted RNA affinity (Crooke et al., 2021). Moreover, another design of ASOs is RNase H independent or the occupancy-only pathway, which are designed as a steric hindrance to spatially

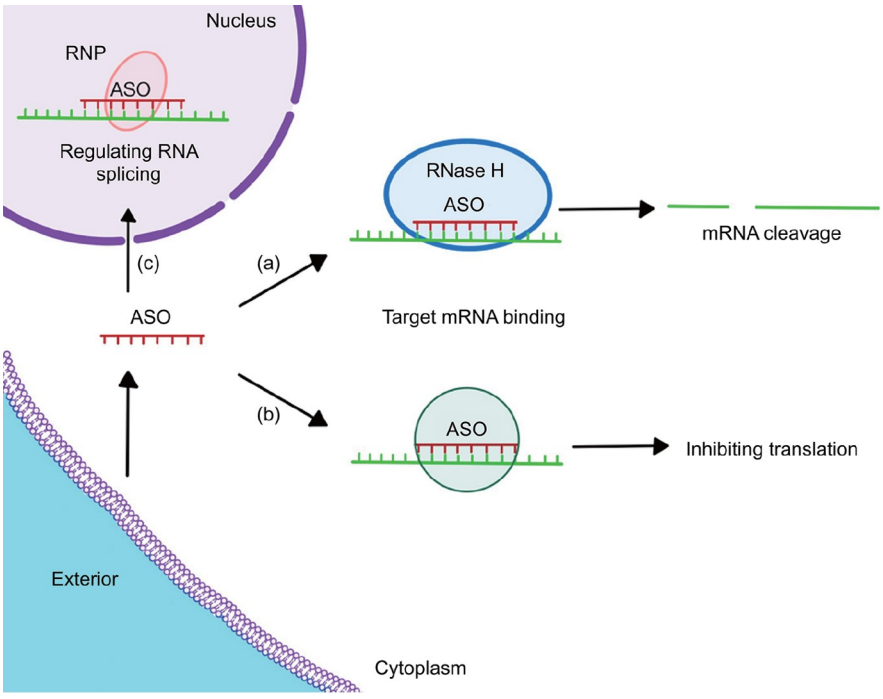

Figure 3 Schematic diagram of ASO's mechanism of action. A, ASOs in the cytoplasm bind to targeted RNA for RNase H cleavage, leading to target mRNA degradation. B, ASOs in the cytoplasm can also bind to mRNA to block RNA binding protein complexes from binding and inhibit target mRNA translation. C, ASOs can enter the nucleus and modulate splicing by binding and blocking a splice junction or exonic/intronic sequences to skip or include targeted exons, which can be catalyzed by the ribonucleoprotein (RNP) complex.

inhibit or prevent translation or splicing of the targeted RNA (Damase et al., 2021).

Several ASO drugs have been approved by the FDA and EMA, as seen in Table 3. To date, there have been nine ASO-based drugs approved for commercial use, all of which treat rare diseases. However, the ASOs currently under development display the application of the ASO platform moving towards treating other common diseases.

The most recently approved ASO-based drug, casimersen, received accelerated approval by the U.S. FDA to treat Duchenne muscular dystrophy (DMD) (Gagliardi and Ashizawa, 2021). DMD is an X-linked recessive degenerative neuromuscular disease caused by frameshift or nonsense mutations in the *DMD* gene encoding the dystrophin protein, which inhibits functional dystrophin production (Shirley, 2021). Casimersen utilizes the PMO-based strategy to cause exon 45 skipping in the *DMD* gene to bypass the frameshift mutations and allow for internally truncated but partially functional dystrophin production (Wagner et al., 2021; Crooke et al., 2021). In its phase 1/2 study, the PMO drug candidate was well tolerated in patients with DMD, which was amenable to exon 45 skipping. Interim results of the phase 3 ESSENCE trial (NCT identifier: NCT02500381) show that all evaluable recipients of casimersen displayed an increase in exon 45 skipping; however, due to its small sample size and ongoing phase 3 ESSENCE trial, it is challenging to determine casimersen's ability to improve motor function (Wagner et al., 2021; Shirley, 2021). The remaining challenge for developing ASO-based drugs to treat neuromuscular diseases is the inadequate distribution and uptake of ASOs by muscle tissue (Crooke et al., 2021).

In addition to rare diseases, cardiovascular diseases are also an area of target for ASOs. Similar to the siRNA therapeutic, ASO drug candidates are also under development to treat CVD. Pelacarsen is a first-in-class GalNAc-conjugated PS ASO that targets the mRNA of apolipoprotein(a) (apo(a)) using RNase H1 dependent cleavage (Crooke et al., 2021). Lipoprotein(a) (Lp(a)) is an LDL-like lipoprotein containing ApoB and apo(a) linked by a disulfide bridge, which has been found associated with increased risk of CVD (Fernandez-Prado et al., 2020). In its phase 2 study (NCT identifier: NCT03070782) of 286 patients with established CVD and increased Lp(a) levels, Tsimikas et al. (2020) observed dose-dependently decreased Lp(a) levels by 35 to 80% with a favorable safety profile after subcutaneous administration of pelacarsen. Given the promising phase 2 results of pelacarsen, the ASO drug candidate for CVD is under phase 3 clinical trial (NCT identifier: NCT04023552).

Advancement in antisense technology pushed the development in ASOs, which demonstrated ASO-based drugs as a versatile and safe therapeutic approach. A large number of ASO candidates treating more common diseases are in late-stage clinical development, which will yield meaningful results in the near future. There are existing challenges for

Table 3 List of representative ASO drugs approved for marketing or are currently in/completed phase 3 clinical trials[a)]

Drug Name	Indication	Developer	Status
Eteplirsen	Duchenne muscular dystrophy (DMD)	Sarepta Therapeutics	FDA approval on 09/19/2016 (N206488**)
Nusinersen	Spinal muscular atrophy (SMA)	Biogen/Ionis	FDA approval on 12/23/2016; EMA approval in 2017 (N209531*; APN: EMEA/H/C/004312)
Inotersen	hATTR	Akcea therapeutics/Ionis	FDA approval on 10/05/2018; EMA approval in 2018 (N211172*; APN: EMEA/H/C/004782)
Golodirsen	DMD	Sarepta therapeutics	FDA approval on 12/12/2019 (N211970*)
Viltolarsen	DMD	NS pharma	FDA approval in 08/12/2020 (N212154*)
Casimersen	DMD	Sarepta therapeutics	FDA approval on 02/25/2021 (N213026*)
Milasen	Batten Disease	Boston Children's Hospital	FDA approval in 2018; Personalized drug developed for one patient
Volanesorsen	Familial Chylomicronemia Syndrome (FCS)	Akcea therapeutics/Ionis	Conditional approval by EMA (APN: EMEA/H/C/004538)
Pelacarsen (TQJ230)	Cardiovascular Disease and Lipoprotein(a)	Novartis/Ionis	Phase 3 (NCT04023552**)
Eplontersen	Transthyretin-Mediated Amyloid Cardiomyopathy (ATTR CM)	Ionis/AstraZeneca	Phase 3 (NCT04136171**, NCT04136184**)
Olezarsen	Familial Chylomicronemia Syndrome	Ionis	Phase 3 (NCT05185843**, NCT05130450**)
Tofersen	Amyotrophic Lateral Sclerosis Associated With a SOD1 Gene Mutation	Biogen, Ionis	Phase 3 (NCT04856982**, NCT02623699**)
Tominersen	Huntingtons Disease	Hoffmann-La Roche, Ionis	Phase 3 (NCT03761849**)
ION363 (Jacifusen)	Amyotrophic Lateral Sclerosis	Ionis	Phase 3 (NCT04768972**)
Donidalorsen	Hereditary Angioedema	Ionis	Phase 3 (NCT05139810**)
Fomivirsen	Cytomegalovirus retinitis (CMV) in immunocompromised patients	Isis pharmaceuticals (now Ionis)	FDA approval in 1998; EMA approval in 1999; Withdrawn in 2006
Mipomersen	Homozygous familial hypercholesterolemia	Kastle therapeutics; Genzyme; Ionis	FDA approval in 2013; Discontinued

a) APN, agency product number; *, application number; **, Clinicaltrials.gov identification number. Data adapted from https://clinicaltrials.gov.

ASOs to overcome to expand their clinical application, such as efficient targeted delivery to other tissue and reducing off-target accumulation in other organs (Dhuri et al., 2020). With the development of novel delivery platforms, there is an optimistic outlook for ASOs to treat more common diseases.

saRNA therapeutics

The family of small dsRNA also includes small activating RNA (saRNA), first reported by Li et al. (2006) and Janowski et al. (2007). The researchers discovered that these 21-nt dsRNAs targeting specific gene promoters induced transcriptional gene activation and was coined RNA activation (RNAa). Both studies described a natural phenomenon of gene expression induction mediated by the small duplex RNA. Li et al. (2006) described the designed 21-nt dsRNA complementary to the promoter region of E-cadherin, p21, and VEGF (vascular endothelial growth factor) genes induced gene expression in a sequence-specific manner and dependency on Ago2, similar to RNAi. Janowski et al. (2007) demonstrated an induced expression of progesterone receptor via complementary duplex RNAs targeting its promoter region. Additionally, RNAa has been shown conserved in a variety of mammalian cells (Huang et al., 2010). There are similarities between the molecular mechanism between RNAi and RNAa, as seen in Figure 4. In RNAa, saRNAs are loaded into Ago2 protein to form the RNA-induced transcriptional activation (RITA) complex. The RITA complex consists of the saRNA/Ago2 complex, RNA helicase A, and RNA polymerase-associated protein CTR9, which interacts with RNA Pol II to trigger transcription initiation and productive elongation (Portnoy et al., 2016). RNAa is distinct in its molecular kinetics, genome-targeting capabilities and activation of target gene transcription elongation in the nucleus (Voutila et al., 2017).

The discovery of RNAa and the role of saRNA offer new insights into the research of selective gene activation. Furthermore, saRNA provides itself as a novel therapeutic modality to upregulate gene expression in diseases with repressed transcriptional or translational activity (Voutila et al., 2017). There are several advantages of developing saRNAs as a therapeutic, including low immunogenicity and locus-specific gene transcription activation; however, its drawbacks like RNase degradation sensitivity and off-target effects are also critical challenges (Ghanbarian et al., 2021).

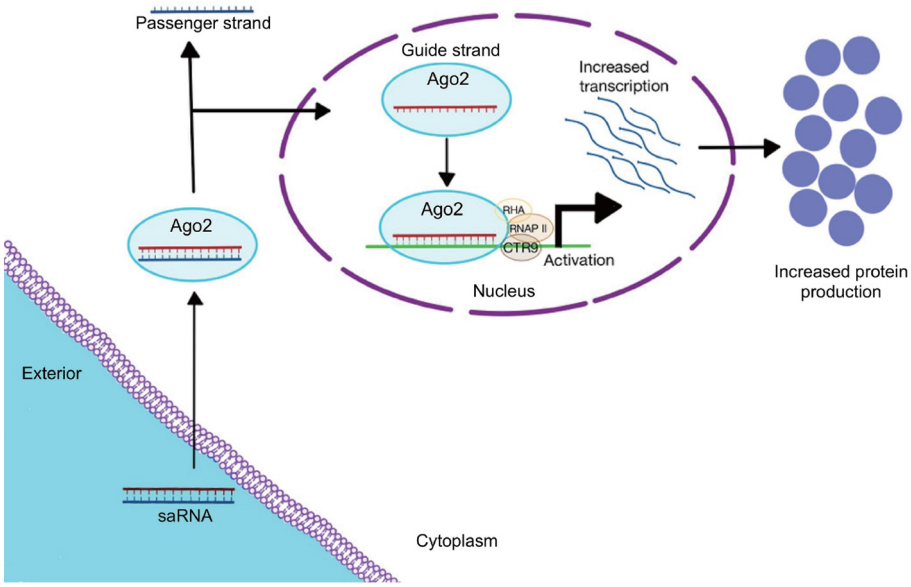

Figure 4 Schematic diagram of saRNA mechanism of action. Introduced saRNA is loaded into the Ago2 protein, which is followed by strand selection cleaved by Ago2 to form the saRNA/Ago2 complex and is translocated into the nucleus. The active RITA complex targets and binds to the promoter region of the genomic target locus and associates with RHA and CTR9, inducing transcriptional activity mediated by RNA Polymerase II (RNAP II).

Currently, several saRNA drug candidates are under development, as seen in Table 4. Although most candidates are still in the preclinical stage, MTL-CEBPA developed by MiNA Therapeutics has entered phase 2 with the combination of the protein kinase inhibitor, sorafenib, to treat patients with advanced hepatocellular carcinoma (HCC) as a result of hepatitis B or C infection (NCT identifier: NCT04710641).

MTL-CEBPA is a first-in-class saRNA oligonucleotide with 2′-O-Me modification, encapsulated by SMARTICLES liposomal nanoparticles, and administered by intravenous infusion to treat patients with HCC (Sarker et al., 2020). The transcription factor C/EBP-a is a leucine zipper protein that primes and activates myeloid gene expression via promoter region binding of the myeloid gene, which is known to be associated with hepatocyte regulation. In murine tumor models, researchers found downregulation of C/EBP-a in myeloid-derived suppressor cells; and upregulation of C/EBP-a inhibited tumor growth in liver cancer rodent models (Reebye et al., 2018; Sarker et al., 2020). MTL-CEBPA is designed to upregulate C/EBP-a by inducing transcription of the *CEBPA* gene. Results of the first-in-human phase 1 study of MTL-CEBPA conducted in patients with HCC associated with cirrhosis, nonalcoholic steatohepatitis, or with liver metastases displayed an acceptable safety profile of the saRNA drug candidate with an initial recommended dose of 130 mg m^{-2} and a reported target engagement displayed by its pharmacodynamic analysis. However, its efficacy profile is limited: among the 24 patients evaluable for the efficacy of MTL-CEBPA as monotherapy, only one patient was observed with objective tumor response and partial response in two years. With the administration of tyrosine kinase inhibitors (sorafenib, lenvatinib, or regorafenib) to seven patients, three were observed with a complete response, two with stable disease, and one with partial response (Sarker et al., 2020). The phase 1 study concludes that pretreatment of MTL-CEBPA for HCC contributes to creating a more receptive tumor microenvironment for the therapeutic effect of tyrosine kinase inhibitors, like sorafenib (Sarker et al., 2020). In addition to MTL-CEBPA plus sorafenib in phase 2, MiNA Therapeutics is also developing MTL-CEBPA with the PD1 checkpoint inhibitor, pembrolizumab, in patients with advanced solid tumors, which entered phase 1 (NCT identifier: NCT04105335).

Although the phase 1 clinical results of MTL-CEBPA are promising, there are remaining unclarity and challenges faced by this novel oligonucleotide therapeutic modality. A clear mechanistic understanding is still lacking in terms of target sequence identification; the accessibility of target genomes when additional DNA elements are involved is also unclear (Kingwell, 2021).

CircRNA therapies

In addition to the RNA therapies described, another emerging RNA therapeutic gained attention as significant research progress has been made. Circular RNAs (circRNAs) are a class of ring structured non-coding RNAs with the absence of end motifs, such as the 5′ cap and 3′ poly(A) tails, mainly endogenous to eukaryotic cells (Chen and Yang, 2015). First discovered in viroids by Sanger et al. in 1976, circRNAs are now found to be highly conserved across nu-

Table 4 List of representative saRNA therapeutics candidates in clinical development[a)]

Drug Name	Indication	Developer	Status
MTL-CEBPA plus sorafenib	Hepatocellular carcinoma (HCC) as a result of Hepatitis B, Hepatitis C	MiNA therapeutics	Phase 2 (NCT04710641*)
MTL-CEBPA plus pembrolizumab	Advanced solid tumors	MiNA therapeutics	Phase 1 (NCT04105335*)
RAG-01	Bladder cancer	Ractigen	Preclinical
RAG-06	Spinal muscular atrophy (SMA)	Ractigen	Preclinical

a)*, Clinicaltrials.gov identification number. Data adapted from Kingwell (2021).

merous species through RNA sequencing (Sanger et al., 1976; Kristensen et al., 2019). Due to the circular character of circRNAs, their stability can be superior to linear RNAs as they are resistant to degradation by various RNA exonucleases (Jeck et al., 2013). In addition, Wesselhoeft et al. (2018) demonstrated the robust protein expression of exogenous circRNAs for longer durations compared to unmodified and modified linear RNAs. Therefore, circRNAs provide promising medical applications given their stability and needless of modifications for expression in eukaryotic cells.

Unlike most linear RNA, the biogenesis of circRNA involves backsplicing of exons, which ligates the downstream "tail" (3′) to the upstream "head" (5′) via covalent bonding. This covalent bond formation gives rise to circRNA's natural ability to withstand major RNA decay pathways (Jeck and Sharpless, 2014; Vicens and Westhof, 2014; Wang and Wang, 2015). Given the exceptional stability of circRNA, its regulation and turnover are significant in managing its abundance. Recently, more research elucidated the turnover mechanism of circRNA in which specific circRNAs, like CDR1as, can be degraded through perfect complementary miRNA binding followed by Ago2-mediated cleavage; RNase L in response to viral infection can also degrade circRNAs; highly structured circRNA can be regulated via structure-mediated RNA decay (Hansen et al., 2011; Liu et al., 2019; Fischer et al., 2020). That being said, additional studies are warranted to determine the molecular pathways of circRNA.

Like other RNA therapeutics, circRNA emerges as a potential therapy to regulate gene expression or carry modular effects. Synthetic circRNAs have been successfully shown to have a strong and stable translation in eukaryotic cells. Wesselhoeft et al. (2018) designed self-splicing precursor RNA to optimize circularization efficiency using spacer sequences and were able to circularize various lengths of RNAs up to 5 kb. The engineered HPLC-purified circRNAs were found to have a translation efficiency of up to 97% compared with the control group. Furthermore, such purified circRNA can evade cellular RNA sensors, such as toll-like receptors and RIG-I, while providing more stable protein expression than unpurified circRNA (Wesselhoeft et al., 2019). Additionally, circRNA can also be formulated with LNP for effective *in vivo* delivery and translation in mice and rhesus macaques (Wesselhoeft et al., 2019; Qu et al., 2022). Recently, Qu et al. (2022) presented a circRNA vaccine against SARS-CoV-2 encoding the trimeric RBD of the spike protein, which provided adequate protection against SARS-CoV-2 in animals. Moreover, they also demonstrated the use of synthetic circRNAs to express SARS-CoV-2 neutralizing antibodies and hACE2 decoys to neutralize pseudovirus particles (Qu et al., 2022).

Finally, another application of engineered circRNA is RNA editing, in which circular ADAR-recruiting RNAs were used to recruit native ADAR1 or ADAR2 enzymes to alter a specific adenosine base to inosine to perform precise endogenous RNA editing (Qu et al., 2019; Yi et al., 2022). In addition to engineered circRNAs, another approach to circRNA therapeutics is the utilization of circRNA-based aptamers in which an expression system called twister-optimized RNA for durable overexpression (Tornado) performed RNA circularization and produced RNA aptamers capable of protein binding (Litke and Jaffrey, 2019). Although notable progress has been made in the research and application of circRNAs, most candidates are currently under the discovery stage or preclinical development. As of the date of this paper, no circRNA therapeutic candidate has entered clinical trials. CircRNA presents several promising medical and research applications, from therapeutic drugs and protein replacement therapies to prophylactic vaccines. It is also important to note that another potential therapeutic approach of circRNA is to target native circRNAs for modulation using other methods, such as CRISPR-Cas9 or siRNA, as well as using native circRNAs for biomarking or sponging of varying diseases, including cancer, cardiovascular diseases, and neurological diseases (He et al., 2021; Tian et al., 2021; Zhang et al., 2018). Lastly, many challenges remain for developing synthetic circRNAs as a therapeutic agent, such as controlling the expression level of circRNA to avoid sustained overexpression due to its exceptional stability, large-scale manufacturing of highly purified artificial circRNAs, and targeted delivery of circRNAs. Therefore, further research accompanied by clinical studies may address and overcome these challenges. Nevertheless, understandings from linear RNA therapeutics, like mRNA-based therapies, can be translatable to circRNA-based ther-

apeutics and provide valuable insights for the development of circRNAs as a therapeutic agent.

mRNA-based therapeutics

Unlike the previously described RNAs, messenger RNA (mRNA), a critical molecule of life, is a single-stranded RNA complementary to the antisense strand of DNA. As its name indicates, mRNA is the messenger between the protein-encoding DNA translation in the nucleus and protein production in the cytoplasm (Pardi et al., 2018). Given the vital role of mRNA in protein production as the intermediator of the central dogma of molecular biology, several therapeutic strategies are developed for this new class of drug, including mRNA-based vaccines and mRNA replacement therapy. Wolff et al. (1990) first described the successful introduction of *in vitro* transcribed (IVT) mRNA in animals. Significant progression in the field was made over the past decade to enable mRNA therapeutics as a promising modality for diseases, such as infectious diseases and cancer. The mRNA-based therapy approach presents itself with many advantages, such as the relatively low risk of insertion mutagenesis and no requirement of entering the nucleus for functionality.

Additionally, the transient nature of mRNA provides benefits for more temporary expression of protein when needed (Granot and Peer, 2017). By introducing chemically modified mRNAs into the cytoplasm of cells, genetic diseases with reduced or suppressed levels of such protein may be expressed to resemble the natural protein (Lieberman, 2018; Feng et al., 2021). Furthermore, such mRNAs can also be used as a prophylactic vaccine, in which the mRNA can encode for specific foreign antigens to evoke protective immunity against infectious diseases; or to prime the immune system to stimulate cell-mediated responses to target tumors as a therapeutic vaccine (Damase et al., 2021).

mRNA vaccines

Vaccination prevents illness and is one of the most effective public health interventions to prevent the spread of infectious diseases. The widespread use of vaccines led to the complete eradication of many infectious diseases and reduced incidences of polio, measles, and other diseases worldwide (Maruggi et al., 2019). In the past centuries, vaccinology evolved with the appearance of conventional vaccine approaches, such as live attenuated and inactivated vaccines, providing durable protection against an array of diseases. Although there are advancements in conventional vaccine approaches, there are remaining challenges in vaccine development to meet current medical needs, such as the emergence of infectious pathogens with a superior ability to evade adaptive immune responses or demands cellular immune responses. In addition, with emerging viruses, there is a necessity for a vaccine approach that enables rapid development and large-scale production with desirable properties for wide-ranging distribution (Pardi et al., 2018).

Moreover, vaccines for non-infectious diseases are also needed, where conventional vaccine approaches may not be appropriate. Therefore, developing a more adaptable and potent vaccine platform is critical. Recent advancements in mRNA vaccines, such as mRNA sequence engineering, large-scale production development, and efficient delivery methods, further accelerated the development of this vaccine platform (Pardi et al., 2020).

Prophylactic vaccine

As introduced previously, mRNA vaccines can be used for prophylactic or therapeutic purposes. Recent advancements in design, manufacturing and delivery methods of mRNAs have made mRNA vaccines an attractive alternative to conventional approaches. For prophylactic purposes, the synthetic mRNA encoding for antigens is delivered to the cytoplasm, which, when expressed, elicits an immune response without crossing the nuclear membrane barrier, as seen in Figure 5. There are two major types of IVT mRNAs, which both mimic endogenous mRNA structure: non-replicating mRNA and self-amplifying mRNA. The former's structure consists of a 5′ cap, 5′ untranslated region (UTR), an open reading frame encoding the antigen, 3′ UTR, and a polyA tail; self-amplifying mRNA also contains viral replication machinery like the replicase gene to express RNA-dependent RNA polymerase, which allows for intracellular RNA amplification and ample antigen expression (Pardi et al., 2018; Chaudhary et al., 2021).

Transcribed from the DNA template, mRNA carries genetic information to guide the translation and production of intracellular proteins, membrane proteins, and extracellular proteins. Similarly, the core principle of mRNA vaccines is to deliver mRNA encoding information, such as antigen proteins, to be translated in the cell. Thereby, effectively activating cellular immunity and humoral immunity, as seen in Figure 6.

In addition to the similarity to endogenous mRNA, mRNA vaccines can also express complex antigens without packaging restrictions. The cell-free manufacturing process utilizing gene sequence information allows rapid and scalable production to quickly respond to any emerging infectious disease (Maruggi et al., 2019). Over years of research and investment in mRNA therapeutics, many challenges in its pharmacology, such as stability and efficacy, were resolved (Sahin et al., 2014; Chaudhary et al., 2021).

With the outbreak of the severe acute respiratory syndrome

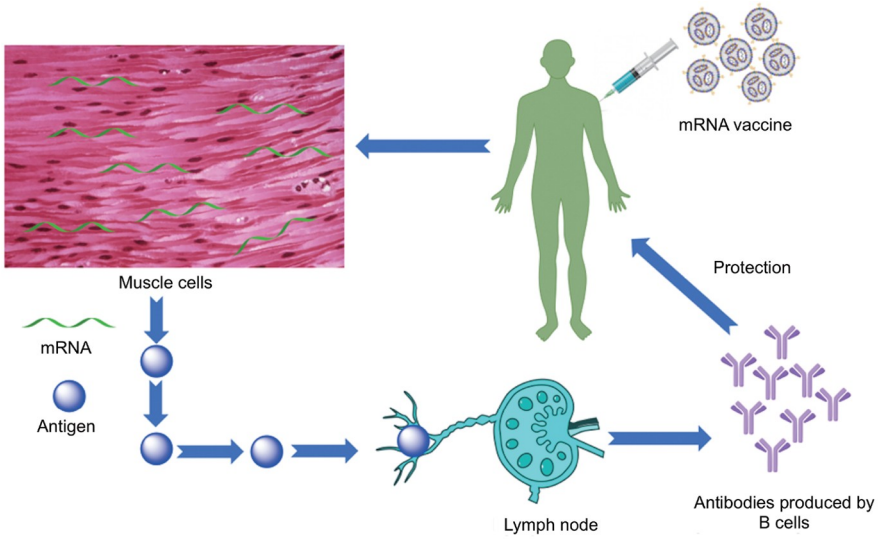

Figure 5 Schematic diagram of mRNA vaccine mechanism of action. The mRNA vaccine contains LNP-encapsulated mRNA sequences (green), which are injected via intramuscular injection. The encapsulated mRNA encoding antigen of interest (blue) are taken up by muscle cells and are expressed in the cytoplasm of the cell. The endogenously produced antigens are secreted and are carried to local lymph nodes, which stimulates both cell-mediated immunity and humoral immunity, such as antibody (purple) secretion by differentiated B cells. The mRNA vaccine approach provides protection through active immunization without containing the part of an actual virus or bacteria.

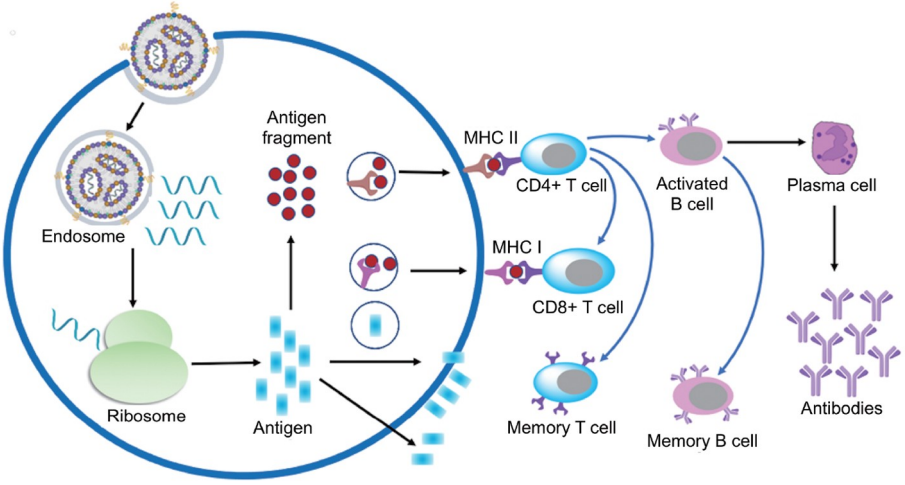

Figure 6 Schematic diagram of mRNA vaccine evoking immunity in antigen-presenting cells (APCs). Following endocytosis of mRNA vaccine by APC, the mRNA is translated into an antigenic peptide by the ribosome and stimulates cellular immune responses. The proteasome complex processes the antigenic peptide into smaller peptide epitopes which can be presented on the cell surface via major histocompatibility complex (MHC) class I or class II depending on the type of APC. The MHC class I presented epitopes are recognized by $CD8^+$ cytotoxic T cells for activation to kill infected cells. The MHC class II presented epitopes are recognized by $CD4^+$ helper T cells, which facilitate B cell activation and neutralizing antibody production and initiate humoral immune responses through phagocytes.

coronavirus 2 (SARS-CoV-2) pandemic and the release of its complete genome in January 2020, the race to develop an effective vaccine against SARS-CoV-2 commenced. As a result, numerous vaccines were developed against SARS-CoV-2, including several RNA-based vaccines, as seen in Table 5. Spikevax developed by Moderna and Comirnaty developed by Pfizer and BioNTech quickly received Emergency Use Authorizations (EUA) by the FDA and conditional marketing authorizations (CMA) by the EMA. Currently, only Comirnaty is approved by the FDA. The mRNA vaccines demonstrated their high efficacy and ideal safety profile, which were developed and administered at an unprecedented rate. Furthermore, the development of these vaccines validated the mRNA-based platform and sparked immense attraction to the application of mRNA therapeutics.

Coronavirus 2019 (COVID-19) is a highly contagious viral infectious disease caused by the virus SARS-CoV-2, which has caused a catastrophic impact on the world as a global health crisis. In response to the global pandemic, comirnaty (BNT162b2 or tozinameran) was developed by Pfizer and BioNTech and was the first FDA-approved mRNA vaccine for prophylactic indications. The comirnaty vaccine is a

Table 5 List of RNA-based vaccines against COVID-19 in clinical trials or approved[a)]

Vaccine Name	Dose; number of doses (as reported in its corresponding clinical trial listed in Status column)	Developer	Status
Comirnaty (BNT162b2, tozinameran)	30 μg; 2 doses	Pfizer/BioNTech	FDA approval in 08/23/2021 (STN: 125742); CMA received on 12/21/2020 (APN: EMEA/H/C/005735)
Spikevax (mRNA-1273, elasomeran, TAK-919)	100 μg; 2 doses	Moderna, National Institute of Allergy and Infectious Diseases (NIAID)	EUA received on 12/18/2020; CMA received on 06/01/2021 (APN: EMEA/H/C/005791)
CVnCoV	12 μg; 2 doses	CureVac	Phase 3 withdrawn (NCT04838847*)
LUNAR-COV19 (ARCT-154)	5 μg; 2 doses	Arcturus Therapeutics	Phase 3 (ISRCTN15779782; NCT05012943*)
ARCoV	15 μg; 2 doses	Academy of Military Science, Yuxi Walvax Biotechnology, Suzhou Abogen Biosciences	Phase 3 (NCT04847102*)
DS-5670a	10, 30 or 60 μg; NR	Daiichi Sankyo	Phase 2/3 (jRCT2071210106)
mRNA-1273.211. (A multivalent booster candidate combining mRNA-1273 plus mRNA-1273.351.)	50 or 100 μg; 1 dose	Moderna	Phase 2/3 (NCT04927065*)
ARCT-021	5.0 μg or 7.5 μg; 1 or 2 doses	Arcturus Therapeutics	Phase 2 (NCT04668339*)
PTX-COVID19-B	40, 60, or 80 μg; 2 doses	Providence Therapeutics	Phase 2 (NCT05175742*)
MRT5500	NR; NR	Sanofi Pasteur and Translate Bio	Phase 2 (NCT04798027*)
EXG-5003	NR; NR	Elixirgen Therapeutics	Phase 1/2 (NCT04863131*)
RBMRNA-176	NR; 2 doses	Argorna Pharmaceuticals/RiboBio	Phase 1/2 (ChiCTR2200057780*)
ARCT-165	NR; 2 doses	Arcturus Therapeutics	Phase 1/2 (NCT05037097*)
LNP-nCoVsaRNA	0.1 μg, 0.3 μg, or 1.0 μg; 2 doses	Imperial College London	Phase 1 (ISRCTN17072692)
ChulaCov19	10 μg, 25 μg, or 50 μg; 2 doses	Chulalongkorn University	Phase 1 (NCT04566276*)
CoV2 SAM (LNP) vaccine	1 μg; 2 doses	GlaxoSmithKline	Phase 1 (NCT04758962*)
HDT-301	1 μg or 5 μg; 2 doses	SENAI CIMATEC	Phase 1 (NCT04844268*)
mRNA-1283	NR; 2 doses	Moderna	Phase 1 (NCT04813796*)
COVID-19 mRNA vaccine	25 μg or 45 μg; NR	Stemirna Therapeutics, Shanghai East Hospital	Phase 1 (NCT05144139*)
LNP-nCOV saRNA-02 vaccine	5.0 μg; 2 doses	MRC/UVRI and LSHTM Uganda Research Unit	Phase 1 (NCT04934111*)
HDT-301 vaccine	NR; 1-2 doses	HDT Bio	Phase 1 (NCT05132907*)
VLPCOV-01	0.5 mL; NR	VLP Therapeutics Japan GK	Phase 1 (jRCT2071210067)

a) STN, submission tracking number; APN, agency product number; NR, not reported. *, Clinicaltrials.gov identification number. Data adapted from WHO COVID-19 Vaccine Tracker and Landscape.

nucleoside-modified mRNA encoding the SARS-CoV-2 spike (S) protein and is encapsulated by lipid nanoparticles, allowing the expression of S antigens. The SARS-CoV-2 S protein is a class I fusion glycoprotein, a major surface protein on the virus and the main target of neutralizing antibodies. S proteins are critical to the virus and its infection because it is capable of marked structural rearrangement, which fuses the cellular membranes of the virus and the host cell to deliver its viral genome into the target host cells for infection (Corbett et al., 2020; Khehra et al., 2021). Comirnaty encodes for the prefusion stabilized and membrane-anchored full-length S protein of SARS-CoV-2, which conserves the neutralization-sensitive epitopes for effectively eliciting an immune response against the S antigen (Polack et al., 2020).

Comirnaty displayed high vaccine efficacy and a good safety profile in its clinical trials. For its phase 2/3 trial, 37,706 participants over the age of 16 were enrolled with a two-dose regimen of 30 μg per dose administered 21 days apart, in which they observed a 52% vaccine efficacy against COVID-19 after one dose, indicating preliminary protection against disease onset; and a 91% vaccine efficacy against COVID-19 seven days after two doses. Overall, a 95% vaccine efficacy was observed among all participants in this phase 2/3 trial. Furthermore, the favorable safety profile was also confirmed in this study, where reactogenicity events, such as fatigue, systemic reactions, and lymphadenopathy, resolved on its own within a few days of onset (Polack et al., 2020). In its previous phase 1/2 study of BNT162b2, immunogenicity was evaluated in adult participants, in which

the same dosing regimen and schedule elicited robust serum SARS-CoV-2 neutralization titers in participants seven days after dose 2 that was persistent one month after dose 2 (Haranaka et al., 2021). Finally, a study conducted utilizing data collected from a mass vaccination campaign in Israel with 3,159,136 participants administered two doses of BNT162b2 found consistent results with the randomized trials: 94% effective in symptomatic COVID-19 prevention; 87% effective in hospitalization prevention; and 92% effective in preventions of severe COVID-19 onset (Dagan et al., 2021). Therefore, the mRNA vaccine, BNT162b2, exemplifies mRNA prophylactic vaccines as an effective platform to protect against infectious diseases while displaying a favorable balance between reactogenicity and immunogenicity.

As an RNA virus, SARS-CoV-2 is susceptible to genetic mutations as it evolves, allowing mutant variants with dissimilar attributes to arise, compared to the wild-type strain. Multiple preliminary studies on BNT162b2 vaccine against various variants, particularly the highly transmissible B.1.617.2 (Delta) and B.1.1.529 (Omicron) variants prevalent around the globe. Tang et al. (2021) assessed the real-world effectiveness of BNT162b2 against the Delta variant with a matched test-negative case-control study and concluded reduced vaccine effectiveness of 51.9% against preventing symptomatic or asymptomatic COVID-19. Additionally, another preliminary laboratory study by Nemet et al. (2022) found the neutralization efficacy against Delta and Omicron significantly reduced with two doses of BNT162b2 and lowered protection effectiveness against these variants. It is important to note that these are preliminary results, and further research is needed to examine the full effect of BNT162b2 on specific variants. That being said, these results indicate a need for vaccine adaptation against emerging variants, which is possible for the RNA-based vaccine approach with its rapid and scalable production (Maruggi et al., 2019). Other than COVID-19 mRNA vaccines, there are also mRNA vaccines developed against other infectious diseases, such as respiratory syncytial virus (RSV), HIV-1, influenza, and Zika virus, which have shown promising preclinical and clinical results (Chaudhary et al., 2021). The approval and emergency authorization of BNT162b2 and mRNA-1273 provided abundant evidence of the safety and efficacy of the mRNA vaccine approach, which accelerated the development of this vaccine technology and gives an optimistic outlook to transform the modern vaccine strategy.

Therapeutic vaccine

In addition to mRNA prophylactic vaccines, mRNA-based vaccines also have the application of cancer immunotherapy. mRNA cancer vaccine encodes for cancer antigens, such as tumor-associated self-antigens (TAA) or tumor-specific antigens (TSA), to induce tumor-specific T-cell response for tumor rejection. The mRNA-based approach is advantageous for cancer immunotherapy because of its antigen delivery and expression abilities, as well as its adjuvant function of innate immunity activation through mRNA design (Linares-Fernández et al., 2020; Beck et al., 2021). There are two approaches to mRNA-based cancer vaccines: mRNA dendritic cell (DC) vaccines and mRNA direct cancer vaccines. mRNA-based DC vaccines involve loading TAAs in DCs *ex vivo* as DCs have the ability to present TAAs and initiate potent effector response against the tumor. The manipulation of DCs *ex vivo* requires hematopoietic progenitor cells isolated from the patient's blood and re-infusion of the transfected cells to the patient, which offers a personalized treatment strategy as the DCs are patient-derived (Perez and De Palma, 2019). However, this process can also be expensive and labor-intensive while adding a layer of complexity. As its name suggests, the latter approach involves a direct injection of mRNA encoding tumor antigens, which can be taken up by local cells for antigen presentation (Beck et al., 2021). Currently, several cancer vaccine candidates are in clinical development, as seen in Table 6.

In a phase 1/2 study by Kyte et al. (2016) (NCT identifier: NCT01278940), melanoma DC vaccines transfected with autologous tumor-mRNA administered alone or with adjuvant interleukin-2 (IL-2) reported elicited tumor-specific T cell immune response associated with improved survival in 16 of 31 patients with advanced melanoma, along with an acceptable safety profile. In another phase 2 study (NCT identifier: NCT03480152), an mRNA direct cancer vaccine encoding 20 different neoantigens expressed by autologous cancer formulated with LNP was administered to four patients with metastatic gastrointestinal cancer. This vaccine candidate was found safe and induced mutation-specific T cell responses against predicted neoepitopes using tumor-infiltrating lymphocytes; however, further clinical efficacy is yet to be determined due to its limited patient number (Cafri et al., 2020). Given the safety, robustness, and relatively lower costs of IVT mRNA technology, personalization of cancer immunotherapy using mRNA vaccines is possible, accompanied by promising preclinical data and ongoing clinical trials (Beck et al., 2021).

In addition to prophylactic and therapeutic mRNA vaccines, the LNP-mRNA technology was recently utilized in a transient antifibrotic chimeric antigen receptor (CAR) T therapeutic in murine models. Rurik et al. (2022) demonstrated that modified nucleoside-containing mRNA encoding CARs designed against fibroblast activation protein (FAP) formulated with CD5-targeted LNPs, also known as targeting antibody/LNP-mRNA cargo, can direct delivery to CD5 cells to express functional CAR T cells.

Table 6 List of representative RNA-based vaccines against cancer currently under or completed Phase 2 or Phase 3 clinical trials[a)]

Intervention	Indication	Developer	Status
Autologous Dendritic Cells loaded with autologous Tumor RNA	Uveal Melanoma	University Hospital Erlangen	Phase 3 (NCT01983748*)
AGS-003 plus standard treatment	Advanced Renal Cell Carcinoma, Renal Cell Carcinoma, Metastatic Renal Cell Carcinoma	Argos Therapeutics	Phase 3 Terminated (NCT01582672)
Dendritic cell immunization plus adjuvant temozolomide	Glioblastoma	Oslo University Hospital	Phase 2/3 (NCT03548571*)
mRNA-4157 plus pembrolizumab	Melanoma	Moderna, Merck Sharp & Dohme	Phase 2 (NCT03897881*)
BNT113 plus pembrolizumab	Unresectable Head and Neck Squamous Cell Carcinoma, Metastatic Head and Neck Cancer, Recurrent Head and Neck Cancer	BioNTech	Phase 2 (NCT04534205*)
DC vaccine	Acute Myeloid Leukemia	Zwi Berneman, University Hospital, Antwerp	Phase 2 (NCT01686334*)
BNT111 plus cemiplimab	Melanoma Stage III, Melanoma Stage IV, Unresectable Melanoma	BioNTech, Regeneron	Phase 2 (NCT04526899*)
RO7198457 plus pembrolizumab	Advanced Melanoma	Genentech, BioNTech	Phase 2 (NCT03815058*)
RO7198457	Colorectal Cancer Stage II, Colorectal Cancer Stage III	BioNTech	Phase 2 (NCT04486378*)
GRNVAC1	Acute Myelogenous Leukemia	Asterias Biotherapeutics	Phase 2 (NCT00510133*)
pp65-shLAMP DC with GM-CSF/ pp65-flLAMP DC with GM-CSF	Glioblastoma Multiforme, Glioblastoma, Malignant Glioma, Astrocytoma, Grade IV GBM	Immunomic Therapeutics	Phase 2 (NCT02465268*)
GEN1046 plus pembrolizumab	Metastatic Non-small Cell Lung Cancer (NSCLC)	Genmab, BioNTech	Phase 2 (NCT05117242*)

a) *, Clinicaltrials.gov identification number. Data adapted from ClinicalTrials.gov.

FAP is a cell-surface glycoprotein expressed in active tissue remodeling and post-acute myocardial infarction injury. Given the robust expression of FAPs in cardiac fibroblasts, it is a viable target and marker for activated cardiac fibroblasts (Aghajanian et al., 2019). In a series of their proof-of-concept experiments, Rurik et al. (2022) successfully demonstrated that modified mRNA encapsulated in targeted LNPs are capable of delivery to specific cell types to produce functional engineered T cells *in vivo*. Furthermore, such delivery produced transient and effective antifibrotic CAR T cells that exhibited trogocytosis. In hypertensive murine models of cardiac injury and fibrosis, 58% of $CD3^+$ T cells were $FAPCAR^+$, suggesting successful transduction of FAPCAR mRNA in CD5/LNP-FAPCAR-injected mice. Finally, treated mice with CD5/LNP-FAPCAR showed improved cardiac function and decreased interstitial fibrosis (Rurik et al., 2022). With LNPs targeted to specific cell types, modified mRNA therapeutics will likely expand its range of application, creating the possibility for a scalable and relatively inexpensive universal therapeutic with the ability of engineered immune functions.

mRNA as protein replacement therapy

Another straightforward application of IVT mRNA is protein replacement therapy, in which IVT mRNA encodes for desired proteins and is expressed in target cells for therapeutic purposes. Therefore, diseases characterized by insufficient protein expression or abnormal protein production, such as genetic disorders, may benefit from protein replacement therapy (Hajj and Whitehead, 2017). The advantage of mRNA-based protein replacement therapies is their ability to express virtually any protein, including secretory proteins, intracellular proteins, and transmembrane proteins (Hou et al., 2021). The majority of clinical trials of protein replacement therapies focus on genetic metabolic disorders, as seen in Table 7.

In the clinical studies of protein replacement therapies, inherited metabolic disorders are of focus due to their characterization of essential enzyme deficiency in which the lack of the enzyme leads to excessive metabolites that result in clinical manifestations (Hou et al., 2021). The clinical data of the above candidates are yet to be published. Therefore, further evidence of the safety and efficacy of protein replacement therapy is needed. In addition to metabolic disorders, the protein replacement therapeutic approach was applied to hematological diseases, like Hemophilia A and B (Ramaswamy et al., 2017; Chen et al., 2020). Ramaswamy et al. (2017) used LNP formulated mRNA encoding human factor IX (hFIX) to treat FIX-deficient mouse model of hemophilia B, which demonstrated the safety and effectiveness of their delivery platform, LUNAR, in mouse models; as well as a therapeutically effective production of FIX proteins delivered to the liver. There are remaining challenges for this

Table 7 List of representative mRNA-based protein replacement therapy under clinical development[a)]

Intervention	Indication	Developer	Status
mRNA-3704	Methylmalonic Acidemia (MMA) Metabolism, Inborn Errors	Moderna	Phase 1/2 Withdrawn (NCT03810690*)
mRNA-3927	Propionic Acidemia	Moderna	Phase 1/2 (NCT04159103*)
MRT5201	Ornithine Transcarbamylase Deficiency	Translate Bio	Phase 1/2 Withdrawn (NCT03767270*)
MRT5005	Cystic Fibrosis	Translate Bio	Phase 1/2 (NCT03375047*)
ARCT-810	Ornithine Transcarbamylase Deficiency	Arcturus Therapeutics	Phase 1 (NCT04442347*, NCT04416126*)

a) *, ClinicalTrials.gov identification number. Data adapted from ClinicalTrials.gov.

modality, such as delivery methods. Currently, the delivery methods of mRNA protein replacement therapies are directed towards the liver, lungs, and the heart. Therefore, delivery to other organs requires further development of delivery strategies (Kowalski et al., 2019).

In addition to delivering mRNAs encoding for absent or abnormal proteins, therapeutics are also developed for LNP formulated with a single guide RNA (sgRNA) and mRNA encoding for CRISPR-associated protein 9 (Cas9). Cas9 is an associated endonuclease capable of double-stranded DNA break and forming a ribonucleoprotein complex by binding with the guide RNA (Qi et al., 2013). By delivering a specific sgRNA complementary to the targeted gene along with the Cas9 mRNA, *in vivo* gene editing can be achieved by which the targeted gene can be cleaved by Cas9 for gene silencing. Multiple drug candidates are under clinical development using the RNA-based CRISPR-Cas9 gene editing strategy to treat genetic disorders. NTLA-2001, developed by Intellia Therapeutics, is a CRISPR-Cas9-based *in vivo* gene editing therapy currently in phase 1 clinical trial (NCT identifier: NCT04601051) to treat transthyretin (ATTR) amyloidosis—a rare, progressive disease characterized by an abnormal buildup of misfolded transthyretin (TRR) protein. The candidate, NTLA-2001, consists of a sgRNA complementary to the targeted TRR gene and a modified mRNA sequence of Cas9 protein formulated with LNP to deliver to the liver. The preclinical studies and first-in-human interim clinical data analysis show decreased concentration of serum TTR in both animal models and patients with hereditary ATTR, demonstrating durable targeted knockout of TTR (Gillmore et al., 2021). These studies yield clinical evidence and proof-of-concept for *in vivo* RNA-based CRISPR-Cas9 gene editing as a promising therapeutic strategy.

Pivotal advancements in the mRNA vaccine field were achieved in the past years, which testified the viability of mRNA-based therapeutics. Progress in manufacturing methods and delivery materials expedited the development of mRNA therapeutics. Although the clinical data of mRNA-based therapeutics are encouraging, remaining challenges in delivery materials for specific cell type targeting and further thorough mechanistic understanding of mRNA therapeutics are required to minimize adverse events further and increase efficacy.

Conclusion and future perspectives

RNA therapeutics is a rapidly emerging field undergoing sweeping expansion. More than fifteen RNA-based therapies have received regulatory approval and more reaching late-stage clinical devolvement. The powerful and versatile platform is capable of addressing many unmet medical needs by current treatments. Because the fundamental challenges of RNA therapies, like delivery, stability, and immunogenicity, have been addressed, the development of RNA drugs is growing rapidly. There is still room for improvement and optimization, like specific cell type delivery, improving endosomal escape, and enhancing potency. However, the continuing discovery and increased mechanistic understanding of RNA activities and delivery platforms provide an optimistic prospect for the RNA therapeutic landscape. The recent successes of GalNAc-siRNAs and mRNA vaccines, accompanied by the potentiality of CRISPR, evince the new era of RNA therapeutics.

References

Aghajanian, H., Kimura, T., Rurik, J.G., Hancock, A.S., Leibowitz, M.S., Li, L., Scholler, J., Monslow, J., Lo, A., Han, W., et al. (2019). Targeting cardiac fibrosis with engineered T cells. Nature 573, 430–433.

Ahmadzada, T., Reid, G., and McKenzie, D.R. (2018). Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. Biophys Rev 10, 69–86.

Alshaer, W., Zureigat, H., Al Karaki, A., Al-Kadash, A., Gharaibeh, L., Hatmal, M.M., Aljabali, A.A.A., and Awidi, A. (2021). siRNA: mechanism of action, challenges, and therapeutic approaches. Eur J Pharmacol 905, 174178.

Asbeutah, A.A.A., Asbeutah, S.A., and Abu-Assi, M.A. (2020). A meta-analysis of cardiovascular outcomes in patients with hypercholesterolemia treated with inclisiran. Am J Cardiol 128, 218–219.

Barquera, S., Pedroza-Tobías, A., Medina, C., Hernández-Barrera, L., Bibbins-Domingo, K., Lozano, R., and Moran, A.E. (2015). Global overview of the epidemiology of atherosclerotic cardiovascular disease. Arch Med Res 46, 328–338.

Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science 289, 905–920.

Beck, J.D., Reidenbach, D., Salomon, N., Sahin, U., Türeci, Ö., Vormehr, M., and Kranz, L.M. (2021). mRNA therapeutics in cancer immunotherapy. Mol Cancer 20, 69.

Brenner, S., Jacob, F., and Meselson, M. (1961). An unstable intermediate carrying information from genes to ribosomes for protein synthesis.

Nature 190, 576–581.
Cafri, G., Gartner, J.J., Zaks, T., Hopson, K., Levin, N., Paria, B.C., Parkhurst, M.R., Yossef, R., Lowery, F.J., Jafferji, M.S., et al. (2020). mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. J Clin Invest 130, 5976–5988.
Chakraborty, C., Sharma, A.R., Sharma, G., and Lee, S.S. (2021). Therapeutic advances of miRNAs: a preclinical and clinical update. J Adv Res 28, 127–138.
Chaudhary, N., Weissman, D., and Whitehead, K.A. (2021). mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov 20, 817–838.
Chen, C.Y., Tran, D.M., Cavedon, A., Cai, X., Rajendran, R., Lyle, M.J., Martini, P.G.V., and Miao, C.H. (2020). Treatment of hemophilia a using factor VIII messenger RNA lipid nanoparticles. Mol Ther-Nucl Acids 20, 534–544.
Chen, L.L., and Yang, L. (2015). Regulation of circRNA biogenesis. RNA Biol 12, 381–388.
Chow, L.T., Gelinas, R.E., Broker, T.R., and Roberts, R.J. (1977). An amazing sequence arrangement at the 5′ ends of adenovirus 2 messenger RNA. Cell 12, 1–8.
Corbett, K.S., Edwards, D.K., Leist, S.R., Abiona, O.M., Boyoglu-Barnum, S., Gillespie, R.A., Himansu, S., Schäfer, A., Ziwawo, C.T., DiPiazza, A.T., et al. (2020). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature 586, 567–571.
Crooke, S.T., Baker, B.F., Crooke, R.M., and Liang, X.H. (2021). Antisense technology: an overview and prospectus. Nat Rev Drug Discov 20, 427–453.
Dagan, N., Barda, N., Kepten, E., Miron, O., Perchik, S., Katz, M.A., Hernán, M.A., Lipsitch, M., Reis, B., and Balicer, R.D. (2021). BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 384, 1412–1423.
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.
de Fougerolles, A., Vornlocher, H.P., Maraganore, J., and Lieberman, J. (2007). Interfering with disease: a progress report on siRNA-based therapeutics. Nat Rev Drug Discov 6, 443–453.
Dhuri, K., Bechtold, C., Quijano, E., Pham, H., Gupta, A., Vikram, A., and Bahal, R. (2020). Antisense oligonucleotides: an emerging area in drug discovery and development. J Clin Med 9, 2004.
Feng, R., Patil, S., Zhao, X., Miao, Z., and Qian, A. (2021). RNA therapeutics-research and clinical advancements. Front Mol Biosci 8.
Fernandez-Prado, R., Perez-Gomez, M.V., and Ortiz, A. (2020). Pelacarsen for lowering lipoprotein(a): implications for patients with chronic kidney disease. Clin Kidney J 13, 753–757.
Fire, A., Xu, S.Q., 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.
Fischer, J.W., Busa, V.F., Shao, Y., and Leung, A.K.L. (2020). Structure-mediated RNA decay by UPF1 and G3BP1. Mol Cell 78, 70–84.e6.
Gagliardi, M., and Ashizawa, A.T. (2021). The challenges and strategies of antisense oligonucleotide drug delivery. Biomedicines 9, 433.
Gallant-Behm, C.L., Piper, J., Lynch, J.M., Seto, A.G., Hong, S.J., Mustoe, T.A., Maari, C., Pestano, L.A., Dalby, C.M., Jackson, A.L., et al. (2019). A microRNA-29 mimic (Remlarsen) represses extracellular matrix expression and fibroplasia in the skin. J Investig Dermatol 139, 1073–1081.
Ghanbarian, H., Aghamiri, S., Eftekhary, M., Wagner, N., and Wagner, K. D. (2021). Small activating RNAs: towards the development of new therapeutic agents and clinical treatments. Cells 10, 591.
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.
Gomez, I.G., MacKenna, D.A., Johnson, B.G., Kaimal, V., Roach, A.M., Ren, S., Nakagawa, N., Xin, C., Newitt, R., Pandya, S., et al. (2015). Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. J Clin Invest 125, 141–156.
Granot, Y., and Peer, D. (2017). Delivering the right message: challenges and opportunities in lipid nanoparticles-mediated modified mRNA therapeutics—an innate immune system standpoint. Semin Immunol 34, 68–77.
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.
Hajj, K.A., and Whitehead, K.A. (2017). Tools for translation: non-viral materials for therapeutic mRNA delivery. Nat Rev Mater 2, 17056.
Hanna, J., Hossain, G.S., and Kocerha, J. (2019). The potential for microRNA therapeutics and clinical research. Front Genet 10.
Hansen, T.B., Wiklund, E.D., Bramsen, J.B., Villadsen, S.B., Statham, A. L., Clark, S.J., and Kjems, J. (2011). miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. EMBO J 30, 4414–4422.
Haranaka, M., Baber, J., Ogama, Y., Yamaji, M., Aizawa, M., Kogawara, O., Scully, I., Lagkadinou, E., Türeci, Ö., Şahin, U., et al. (2021). A randomized study to evaluate safety and immunogenicity of the BNT162b2 COVID-19 vaccine in healthy Japanese adults. Nat Commun 12, 7105.
He, A.T., Liu, J., Li, F., and Yang, B.B. (2021). Targeting circular RNAs as a therapeutic approach: current strategies and challenges. Sig Transduct Target Ther 6, 185.
Hou, X., Zaks, T., Langer, R., and Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. Nat Rev Mater 6, 1078–1094.
Hovingh, G.K., Lepor, N.E., Kallend, D., Stoekenbroek, R.M., Wijngaard, P.L.J., and Raal, F.J. (2020). Inclisiran durably lowers low-density lipoprotein cholesterol and proprotein convertase subtilisin/kexin type 9 expression in homozygous familial hypercholesterolemia. Circulation 141, 1829–1831.
Huang, V., Qin, Y., Wang, J., Wang, X., Place, R.F., Lin, G., Lue, T.F., and Li, L.C. (2010). RNAa is conserved in mammalian cells. PLoS ONE 5, e8848.
Janowski, B.A., Younger, S.T., Hardy, D.B., Ram, R., Huffman, K.E., and Corey, D.R. (2007). Activating gene expression in mammalian cells with promoter-targeted duplex RNAs. Nat Chem Biol 3, 166–173.
Jeck, W.R., and Sharpless, N.E. (2014). Detecting and characterizing circular RNAs. Nat Biotechnol 32, 453–461.
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.
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.
Khan, S.A., Naz, A., Qamar Masood, M., and Shah, R. (2020). Meta-analysis of inclisiran for the treatment of hypercholesterolemia. Am J Cardiol 134, 69–73.
Khehra, N., Padda, I., Jaferi, U., Atwal, H., Narain, S., and Parmar, M.S. (2021). Tozinameran (BNT162b2) vaccine: the journey from preclinical research to clinical trials and authorization. AAPS PharmSciTech 22, 172.
Kristensen, L.S., Andersen, M.S., Stagsted, L.V.W., Ebbesen, K.K., Hansen, T.B., and Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 20, 675–691.
Kingwell, K. (2021). Small activating RNAs lead the charge to turn up gene expression. Nat Rev Drug Discov 20, 573–574.
Kole, R., Krainer, A.R., and Altman, S. (2012). RNA therapeutics: beyond RNA interference and antisense oligonucleotides. Nat Rev Drug Discov 11, 125–140.
Kowalski, P.S., Rudra, A., Miao, L., and Anderson, D.G. (2019). Delivering the messenger: advances in technologies for therapeutic mRNA delivery. Mol Ther 27, 710–728.
Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., and Cech, T.R. (1982). Self-splicing RNA: autoexcision and autocyclization

of the ribosomal RNA intervening sequence of tetrahymena. Cell 31, 147–157.
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.
Kyte, J.A., Aamdal, S., Dueland, S., Sæbøe-Larsen, S., Inderberg, E.M., Madsbu, U.E., Skovlund, E., Gaudernack, G., and Kvalheim, G. (2016). Immune response and long-term clinical outcome in advanced melanoma patients vaccinated with tumor-mRNA-transfected dendritic cells. OncoImmunology 5, e1232237.
Laganà, A., Veneziano, D., Russo, F., Pulvirenti, A., Giugno, R., Croce, C. M., and Ferro, A. (2014). Computational design of artificial RNA molecules for gene regulation. Methods Mol Biol, 393–412.
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-Nucl Acids 4, e252.
Lee, R.C., Feinbaum, R.L., and Ambros, V. (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843–854.
Li, L.C., Okino, S.T., Zhao, H., Pookot, D., Place, R.F., Urakami, S., Enokida, H., and Dahiya, R. (2006). Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci USA 103, 17337–17342.
Li, X., Zhang, J.L., Lei, Y.N., Liu, X.Q., Xue, W., Zhang, Y., Nan, F., Gao, X., Zhang, J., Wei, J., et al. (2021). Linking circular intronic RNA degradation and function in transcription by RNase H1. Sci China Life Sci 64, 1795–1809.
Lieberman, J. (2018). Tapping the RNA world for therapeutics. Nat Struct Mol Biol 25, 357–364.
Linares-Fernández, S., Lacroix, C., Exposito, J.Y., and Verrier, B. (2020). Tailoring mRNA vaccine to balance innate/adaptive immune response. Trends Mol Med 26, 311–323.
Litke, J.L., and Jaffrey, S.R. (2019). Highly efficient expression of circular RNA aptamers in cells using autocatalytic transcripts. Nat Biotechnol 37, 667–675.
Liu, C.X., Li, X., Nan, F., Jiang, S., Gao, X., Guo, S.K., Xue, W., Cui, Y., Dong, K., Ding, H., et al. (2019). Structure and degradation of circular RNAs regulate PKR activation in innate immunity. Cell 177, 865–880.e21.
Maruggi, G., Zhang, C., Li, J., Ulmer, J.B., and Yu, D. (2019). mRNA as a transformative technology for vaccine development to control infectious diseases. Mol Ther 27, 757–772.
Nemet, I., Kliker, L., Lustig, Y., Zuckerman, N., Erster, O., Cohen, C., Kreiss, Y., Alroy-Preis, S., Regev-Yochay, G., Mendelson, E., et al. (2022). Third BNT162b2 vaccination neutralization of SARS-CoV-2 omicron infection. N Engl J Med 386, 492–494.
Otoukesh, B., Abbasi, M., Gorgani, H.O.L., Farahini, H., Moghtadaei, M., Boddouhi, B., Kaghazian, P., Hosseinzadeh, S., and Alaee, A. (2020). MicroRNAs signatures, bioinformatics analysis of miRNAs, miRNA mimics and antagonists, and miRNA therapeutics in osteosarcoma. Cancer Cell Int 20, 254.
Pardi, N., Hogan, M.J., Porter, F.W., and Weissman, D. (2018). mRNA vaccines—a new era in vaccinology. Nat Rev Drug Discov 17, 261–279.
Pardi, N., Hogan, M.J., and Weissman, D. (2020). Recent advances in mRNA vaccine technology. Curr Opin Immunol 65, 14–20.
Patrick Walton, S., Wu, M., Gredell, J.A., and Chan, C. (2010). Designing highly active siRNAs for therapeutic applications. FEBS J 277, 4806–4813.
Perez, C.R., and De Palma, M. (2019). Engineering dendritic cell vaccines to improve cancer immunotherapy. Nat Commun 10, 5408.
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.
Portnoy, V., Lin, S.H.S., Li, K.H., Burlingame, A., Hu, Z.H., Li, H., and Li, L.C. (2016). saRNA-guided Ago2 targets the RITA complex to promoters to stimulate transcription. Cell Res 26, 320–335.
Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., and Lim, W.A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152, 1173–1183.
Qu, L., Yi, Z., Shen, Y., Lin, L., Chen, F., Xu, Y., Wu, Z., Tang, H., Zhang, X., Tian, F., et al. (2022). Circular RNA vaccines against SARS-CoV-2 and emerging variants. Cell 185, 1728–1744.e16.
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.
Raal, F.J., Kallend, D., Ray, K.K., Turner, T., Koenig, W., Wright, R.S., Wijngaard, P.L.J., Curcio, D., Jaros, M.J., Leiter, L.A., et al. (2020). Inclisiran for the treatment of heterozygous familial hypercholesterolemia. N Engl J Med 382, 1520–1530.
Ramaswamy, S., Tonnu, N., Tachikawa, K., Limphong, P., Vega, J.B., Karmali, P.P., Chivukula, P., and Verma, I.M. (2017). Systemic delivery of factor IX messenger RNA for protein replacement therapy. Proc Natl Acad Sci USA 114.
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.
Ray, K.K., Wright, R.S., Kallend, D., Koenig, W., Leiter, L.A., Raal, F.J., Bisch, J.A., Richardson, T., Jaros, M., Wijngaard, P.L.J., et al. (2020). Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med 382, 1507–1519.
Reebye, V., Huang, K.W., Lin, V., Jarvis, S., Cutilas, P., Dorman, S., Ciriello, S., Andrikakou, P., Voutila, J., Saetrom, P., et al. (2018). Gene activation of CEBPA using saRNA: preclinical studies of the first in human saRNA drug candidate for liver cancer. Oncogene 37, 3216–3228.
Ruotsalainen, A.K., Mäkinen, P., and Ylä-Herttuala, S. (2021). Novel RNAi-based therapies for atherosclerosis. Curr Atheroscler Rep 23, 45.
Rupaimoole, R., and Slack, F.J. (2017). MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 16, 203–222.
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.
Sahin, U., Karikó, K., and Türeci, Ö. (2014). mRNA-based therapeutics—developing a new class of drugs. Nat Rev Drug Discov 13, 759–780.
Sanger, H.L., Klotz, G., Riesner, D., Gross, H.J., and Kleinschmidt, A.K. (1976). Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc Natl Acad Sci USA 73, 3852–3856.
Sarker, D., Plummer, R., Meyer, T., Sodergren, M.H., Basu, B., Chee, C.E., Huang, K.W., Palmer, D.H., Ma, Y.T., Evans, T.R.J., et al. (2020). MTL-CEBPA, a small activating RNA therapeutic upregulating C/EBP-α, in patients with advanced liver cancer: a first-in-human, multicenter, open-label, phase I trial. Clin Cancer Res 26, 3936–3946.
Schluenzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., et al. (2000). Structure of functionally activated small ribosomal subunit at 3.3 Å resolution. Cell 102, 615–623.
Segal, M., and Slack, F.J. (2020). Challenges identifying efficacious miRNA therapeutics for cancer. Expert Opin Drug Discovery 15, 987–991.
Setten, R.L., Rossi, J.J., and Han, S.P. (2019). The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov 18, 421–446.
Shirley, M. (2021). Casimersen: first approval. Drugs 81, 875–879.
Sun, H., Krauss, R.M., Chang, J.T., and Teng, B.B. (2018). PCSK9 deficiency reduces atherosclerosis, apolipoprotein B secretion, and endothelial dysfunction. J Lipid Res 59, 207–223.
Tang, P., Hasan, M.R., Chemaitelly, H., Yassine, H.M., Benslimane, F.M.,

Al Khatib, H.A., AlMukdad, S., Coyle, P., Ayoub, H.H., Al Kanaani, Z., et al. (2021). BNT162b2 and mRNA-1273 COVID-19 vaccine effectiveness against the SARS-CoV-2 Delta variant in Qatar. Nat Med 27, 2136–2143.

Tian, T., Zhao, Y., Zheng, J., Jin, S., Liu, Z., and Wang, T. (2021). Circular RNA: a potential diagnostic, prognostic, and therapeutic biomarker for human triple-negative breast cancer. Mol Ther-Nucl Acids 26, 63–80.

Tsimikas, S., Karwatowska-Prokopczuk, E., Gouni-Berthold, I., Tardif, J. C., Baum, S.J., Steinhagen-Thiessen, E., Shapiro, M.D., Stroes, E.S., Moriarty, P.M., Nordestgaard, B.G., et al. (2020). Lipoprotein(a) reduction in persons with cardiovascular disease. N Engl J Med 382, 244–255.

Vicens, Q., and Westhof, E. (2014). Biogenesis of circular RNAs. Cell 159, 13–14.

Voutila, J., Reebye, V., Roberts, T.C., Protopapa, P., Andrikakou, P., Blakey, D.C., Habib, R., Huber, H., Saetrom, P., Rossi, J.J., et al. (2017). Development and mechanism of small activating RNA targeting CEBPA, a novel therapeutic in clinical trials for liver cancer. Mol Ther 25, 2705–2714.

Wagner, K.R., Kuntz, N.L., Koenig, E., East, L., Upadhyay, S., Han, B., and Shieh, P.B. (2021). Safety, tolerability, and pharmacokinetics of casimersen in patients with Duchenne muscular dystrophy amenable to exon45 skipping: a randomized, double-blind, placebo-controlled, dose-titration trial. Muscle Nerve 64, 285–292.

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

Wesselhoeft, R.A., Kowalski, P.S., and Anderson, D.G. (2018). Engineering circular RNA for potent and stable translation in eukaryotic cells. Nat Commun 9, 2629.

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.

Wimberly, B.T., Brodersen, D.E., Clemons Jr, W.M., Morgan-Warren, R.J., Carter, A.P., Vonrhein, C., Hartsch, T., and Ramakrishnan, V. (2000). Structure of the 30S ribosomal subunit. Nature 407, 327–339.

Wolf, D., and Ley, K. (2019). Immunity and inflammation in atherosclerosis. Circ Res 124, 315–327.

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.

Xue, Y., Chen, R., Qu, L., and Cao, X. (2020). Noncoding RNA: from dark matter to bright star. Sci China Life Sci 63, 463–468.

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.

Zamecnik, P.C., and Stephenson, M.L. (1978). Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc Natl Acad Sci USA 75, 280–284.

Zhang, M.M., Bahal, R., Rasmussen, T.P., Manautou, J.E., and Zhong, X.B. (2021a). The growth of siRNA-based therapeutics: updated clinical studies. Biochem Pharmacol 189, 114432.

Zhang, S., Cheng, Z., Wang, Y., and Han, T. (2021b). The risks of miRNA therapeutics: in a drug target perspective. Drug Design, Dev Ther 15, 721–733.

Zhang, Z., Yang, T., and Xiao, J. (2018). Circular RNAs: promising biomarkers for human diseases. EBioMedicine 34, 267–274.