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Biotechnology Methods – Mini-Review

The infinite possibilities of RNA therapeutics

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Abstract: Although the study of ribonucleic acid (RNA) therapeutics started decades ago, for many years, this field of research was overshadowed by the growing interest in DNA-based therapies. Nowadays, the role of several types of RNA in cell regulation processes and the development of various diseases have been elucidated, and research in RNA therapeutics is back with force. This short literature review aims to present general aspects of many of the molecules currently used in RNA therapeutics, including *in vitro* transcribed mRNA (IVT mRNA), antisense oligonucleotides (ASOs), aptamers, small interfering RNAs (siRNAs), and microRNAs (miRNAs). In addition, we describe the state of the art of technologies applied for synthetic RNA manufacture and delivery. Likewise, we detail the RNA-based therapies approved by the FDA so far, as well as the ongoing clinical investigations. As a final point, we highlight the current and potential advantages of working on RNA-based therapeutics and how these could lead to a new era of accessible and personalized healthcare.

Keywords: RNA therapeutics, IVT mRNA, Antisense oligonucleotide, Aptamer, miRNA

Introduction

The recent COVID-19 pandemic has brought to the fore the power of mRNA vaccines and, in this way, has pinpointed the attractiveness of ribonucleic acid (RNA) therapeutics as an exciting new technology in preventing and combatting disease. Thanks to the unique features of this primordial nucleic acid and the confluence of enabling advances in bioinformatics and nanomedicine, a disruptive paradigm of RNA-based drugs is emerging that promises to transform the current standard of healthcare for a wide range of disorders.

RNA is a nucleic acid essential for the development of any form of life and is the genetic material of some viruses. Since its discovery in 1961 (Brenner et al., 1961), the study of RNA has gone through different stages. First, the focus was on the RNA structure and how it was related to DNA; second, the RNA function in protein synthesis and the genetic code; and third, the RNA diversity and its role in complex biological processes. RNA actively participates in protein synthesis, gene expression regulation, modification of other RNA molecules and chemical catalysis, among other processes yet to be defined (Clancy, 2008).

Initially, the central dogma of molecular biology suggested a unidirectional flow of the information in genes, where RNA acts as an intermediary between DNA transcription and protein translation (Crick, 1970). Nevertheless, considering the discovered functions associated with RNA molecules, some researchers deem that RNA had a more crucial role in the evolutionary process of life. According to the RNA world hypothesis, RNA stored genetic information and catalyzed chemical reactions giving rise to primitive cells before the appearance of DNA (Alberts et al., 2002). Although numerous members of the scientific community disagree with this viewpoint, it nonetheless raises questions about the authentic role and importance of RNA in the emergence of life.

Moreover, after years of constant research aided by technological developments, scientists discovered several RNA molecules with diverse roles and others still to be determined. Briefly, Table 1 shows some of the processes in which different types of RNA are involved. Thus, many of these RNAs do not fulfill a single universal function but participate in several biological pathways with similar molecules, which complicate their classification. In general terms, RNA is categorized as coding and noncoding (ncRNA), where messenger RNA (mRNA) constitutes the single member of the first group. The second group, much more extensive, includes RNA molecules that could be classified according to their roles in different biological processes: (i) RNAs more directly related to the translation process, such as ribosomal RNA (rRNA) and transfer RNA (tRNA); (ii) RNAs involved in posttranscriptional modifications or DNA replication including ribonuclease (RNase MRP), ribonuclease P (RNase P), telomerase RNA component (TERC), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), and small Cajal body-specific RNA (scaRNA); and (iii) RNAs with regulatory functions such as microRNAs (miRNAs), small interfering RNAs (siRNAs), P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs), circular RNAs (circRNAs), and long noncoding RNAs (lncRNAs) just to name a few.

The variety and complexity of the different RNA molecules and the constant discovery of new functions associated with them keep alive the debate about how determining RNA was for the origin of the first forms of life and their maintenance until the present. Even more important, they allow us to expand our knowledge about cellular regulation processes and their influence on the development of various disorders, thus opening perspectives into their progress and offering alternatives for their diagnosis, prevention, and treatment, through the burgeoning field of RNA therapeutics.

RNA Therapeutics

In contrast to DNA-based therapeutics which must pass across the cytoplasmic and nuclear membrane and run the risk of

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Table 1. Relevant RNA Molecules in Biological Processes

Type of RNA	Abbreviation	Processes involved	Approximate size (nt)	Reference
Coding RNA				
Messenger RNA	mRNA	Transcription	Variable (depends on protein type and species)	Clancy (2008), Lodish et al. (2000), and Piovesan et al. (2016)
Noncoding RNAs				
Ribosomal RNA	rRNA	Translation	1500–5000	Clancy (2008) and Lodish et al. (2000)
Transfer RNA	tRNA	Translation	70–80	Clancy (2008) and Lodish et al. (2000)
Ribonuclease MRP	RNase MRP	Pre-rRNA processing; DNA replication in mitochondria		Martin and Li (2007) and Woodhams et al. (2007)
Ribonuclease P	RNase P	Pre-tRNA processing; Maturation of lncRNA	500	Abu-Zhayia et al. (2017) and Houser-Scott et al. (2001)
Telomerase RNA component	TR/TER/TERC	Telomerase activity	147–2200	Giardini et al. (2014) and Londoño-Vallejo and Wellinger (2012)
Small nucleolar RNA	snoRNA	Gene expression regulation; Covalent modifications of other RNAs; RNA splicing; rRNA processing; Stress response; Metabolic homeostasis	60–300	Bratkovič et al. (2020), Liang et al. (2019), and Woolard and Chorley (2019)
Small nuclear RNA	snRNA	Intron splicing; Telomere maintaining; Pre-mRNA processing; Transcription factors regulation	150	Hari and Parthasarathy (2019) and Woolard and Chorley (2019)
Small Cajal body-specific RNA	scaRNA	Processing and modification of mRNA, rRNA, tRNA, and snRNAs	-	Xie et al. (2007)
Micro RNA	miRNA	Gene expression regulation; RNA silencing; Cell proliferation and death; Development and function of the immune system; Hematopoietic differentiation; Placental development	19–25	Fu et al. (2013), Lam et al. (2015), Wahid et al. (2010), and Xu et al. (2018)
Small interfering RNA	siRNA	Gene expression regulation; RNA silencing	21–23	Lam et al. (2015), Woolard and Chorley (2019), and Xu et al. (2018)
Piwi-interacting RNA	piRNA	Gene expression regulation; Germ line development; Protection of the genome integrity; Carcinogenesis	26–31	Krishnan and Damaraju (2019) and Woolard and Chorley (2019)
Circular RNA	circRNA	Regulation of transcription and alternative splicing; miRNA and protein inhibition by sequestration; Protein scaffolds for ribonucleoprotein complex formation; Templates for cap-independent translation	Variable	Chen et al. (2020), Huang et al. (2018), and Zhang et al. (2013)
Long noncoding RNA	lncRNA	Regulation of gene expression at transcriptional, posttranscriptional, translational, and posttranslational levels; Chromatin modification and remodeling; Cell differentiation, alternative splicing, and cell cycle regulation	>200	Zhang et al. (2019)

integrating into the host genome, RNA therapeutics can act by attaining the host cytoplasm and there is no potential chromosomal integration (Shin et al., 2018). Currently, the drug market consists mainly of two types of drugs: small molecules and proteins, but the range of disease targets for both proteins or genes of these molecules is limited and unable to meet the required demand (Kaczmarek et al., 2017; Zhou et al., 2019). Therefore, features such as inducing protein coding, inhibiting protein translation, or the specificity to bind to target molecules make some RNA molecules appealing candidates for therapeutic agents (Deweerdt, 2019; Kim, 2020). Moreover, RNA molecules possess a very versatile conformation with the capacity to recognize a diverse range of ligands: small molecules, other nucleic acids, proteins, and cells. Thus, they can act on a variety of known and emerging disease targets that are out of reach for the most established drug models. These features, together with emerging RNA technologies, have motivated researchers to address RNA through different angles, mainly focusing on their therapeutic applications, which is a very promising field (Damase et al., 2021; Dammes & Peer, 2020; Kher et al., 2011; Kim, 2020). Although RNA therapies may be one of the trending topics in present-day research, their initial development dates back to the 90s with a study where mRNA was injected into skeletal muscle leading to the expression of the protein encoded by that RNA (Wolff et al., 1990). The development of RNA therapies was severely hampered for more than two decades by obstacles such as inherent instability of RNA, target delivery,

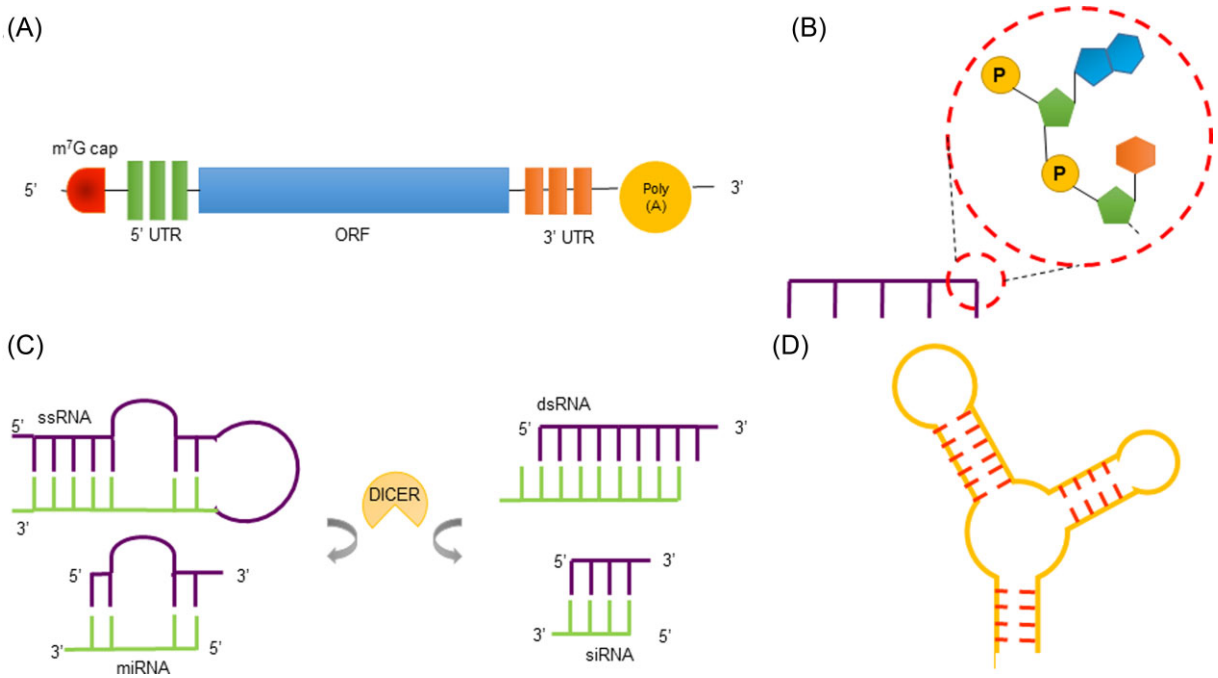

Fig. 1 General structure of different RNA therapeutics. (A) Main components of the IVT mRNA. (B) Antisense oligonucleotide. (C) miRNA (left) and siRNA (right). (D) Aptamer.

potential toxicity, unwanted immune response, and effectiveness (Burnett & Rossi, 2012; Deweerdt, 2019; Kaczmarek et al., 2017; Robinson, 2004). Opportunely over the years, many limitations were overcome, mainly because of the development of new materials and screening technologies for the delivery and structural modification of RNA molecules (Deweerdt, 2019; Kaczmarek et al., 2017; Zhou et al., 2019). Such was the impact of the new chemical modifications and delivery strategies that, in the last 5 years, the FDA approved more than 10 RNA-based drugs, and others are awaiting regulatory decisions or are in the late stages of clinical trials (Chakraborty et al., 2021; Dammes & Peer, 2020; Kim, 2020; Rinaldi & Wood, 2018; Wang et al., 2020). Thus, the recent exponential development of RNA therapies has aroused the interest of many researchers and generated excellent works in this regard (Damase et al., 2021; Dammes & Peer, 2020; Kim, 2020; Wang et al., 2020; Zhou et al., 2019). However, the current rate at which new relevant information is unveiled and more treatments are approved requires constant updating, especially with the growing investment of big pharmaceutical companies to develop therapies to treat and prevent various types of cancer, infectious diseases (such as COVID-19), neurological disorders, heart and metabolic diseases, among others (Chanda et al., 2021; Wang et al., 2020). The objective of this mini-review is to exhibit the state of the art of RNA technologies, synthesizing the most relevant aspects of their manufacturing, delivery, and current applications, and, thus, present to the interested reader a starting point regarding the infinite possibilities of RNA therapeutics.

Types of RNA Therapeutics

Based on the nature of the RNA drugs, RNA therapy can be categorized in two groups. The first category includes RNA drugs that are translated into proteins, for example *in vitro* transcribed mRNA (IVT mRNA). The second category comprises oligonucleotide therapeutics, whose interactions with pre-mRNA, mRNA, or proteins can regulate gene expression in the cell, for example antisense oligonucleotides (ASOs), siRNAs, miRNAs, and RNA aptamers (Fig. 1).

In Vitro Transcribed mRNA

IVT mRNAs are mRNAs produced in a cell-free system and present all essential features that an endogenous mRNA contains. Most IVT mRNA molecules manufactured to date contain the following five elements (Loomis et al., 2016): (i) a 7-methyl guanosine 5′ cap, which initiates mRNA translation, protects mRNA from degradation by exonucleases and avoids induction of the innate immune response; (ii) a 5′ untranslated region (UTR); (iii) an open reading frame (ORF) which codes for antigen (or other protein) of interest; (iv) a 3′ UTR; and (v) a polyadenylated (polyA) tail, which improves mRNA stability, increases mRNA lifespan, enhances mRNA translational activities, and protects mRNA from degradation by nucleases (Fig. 1A). After an IVT mRNA enters the cytoplasm, it is associated with host translational machinery and it produces a peptide that will work as a replacement of a nonfunctional protein or as an antigen, in the case of mRNA vaccines (Miliotou & Papadopoulou, 2020; Wadhwa et al., 2020) (Fig. 2). The first transfection of an IVT mRNA was performed by Wolff and collaborators in 1990, whereby an IVT mRNA was introduced into mouse skeletal muscle leading to a successful intracellular expression (Wolff et al., 1990). Since then, the possible clinical application of IVT mRNA has been considered. Unlike DNA-based cell therapies, IVT mRNA does not need to enter into the nucleus to be active, as once in the cytoplasm it is translated immediately, reducing the risk of integrating into the genome and avoiding any insertional mutagenesis (Jahanafrooz et al., 2021; Sahin et al., 2014).

Oligonucleotide Therapeutics

This category contains single- or double-stranded oligonucleotides produced *in vitro* able to silence or suppress a target through a direct interaction. In 1978, Zamecnik and Stephenson were successful using an ASO to inhibit the production of

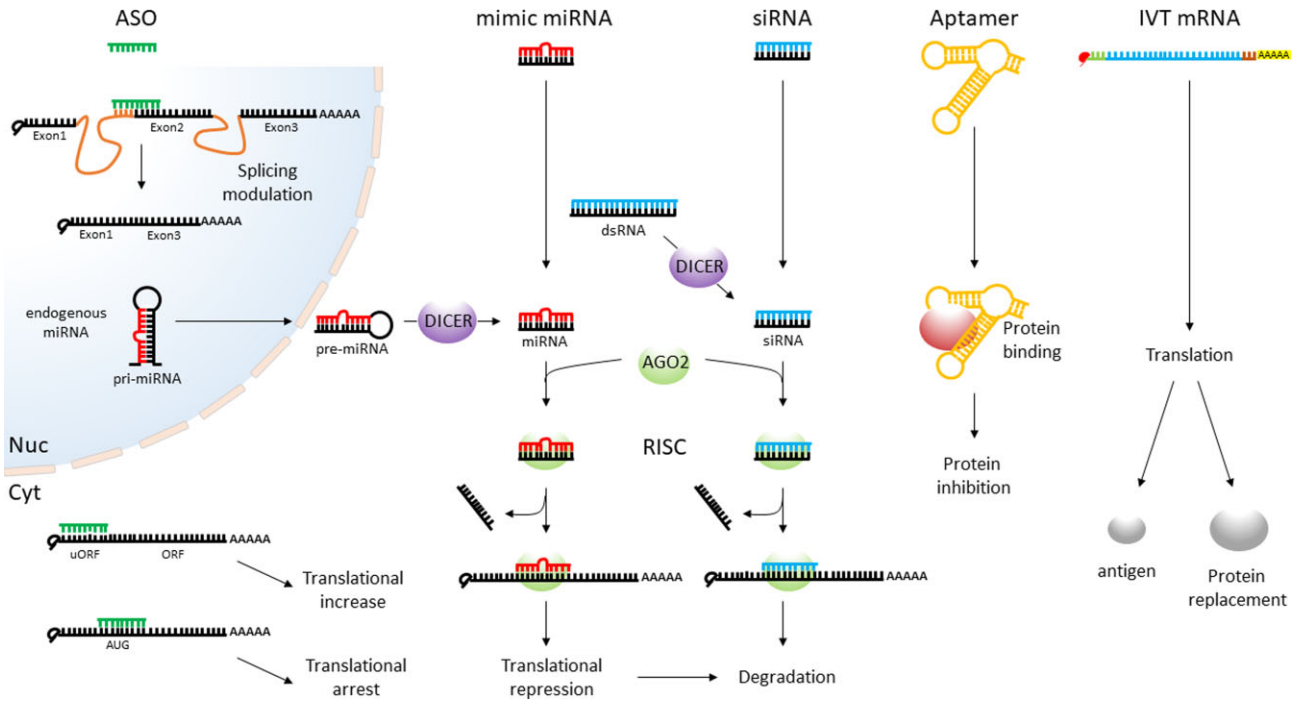

Fig. 2 Mechanisms of action of RNA-based therapeutics. ASOs modulate splicing, increase translation rate, repress translation. Mimic miRNA represses translation and leads to mRNA degradation. siRNA leads to mRNA degradation. RNA aptamer inhibits protein activity. IVT mRNA is translated as a host protein to act as an antigen or to replace a protein in the cell.

the Rous sarcoma virus in chick embryo fibroblasts (Zamecnik & Stephenson, 1978). Initially, the therapeutic potential of oligonucleotides was limited by drawbacks related to their stability, delivery, rapid degradation, and immunogenicity (Glazier et al., 2020). However, the addition of chemical modifications in their backbone structure allowed to improve the action of oligonucleotide therapeutics ASOs (Kher et al., 2011; Kim, 2020; Rinaldi & Wood, 2018). According to Rinaldi and Wood (2018), these modifications include phosphorothioate DNA, phosphorodiamidate morpholino oligomers (PMOs), peptide nucleic acid designs, tricyclo-DNAs (tcDNAs), locked nucleic acid, and ribose substitutions such as 2'-O-methyl (2'-OMe) and 2'-O-methoxyethyl (2'-MOE). In the last decades, with the discovery of the RNA interference pathway, there has been a steady increase in studies of molecules mimicking this mechanism in the cell, for example siRNAs and miRNAs (Dammes & Peer, 2020; Fire et al., 1998; Kher et al., 2011; Khvorova & Watts, 2017; Quemener et al., 2020; Roshan et al., 2009). Finally, therapeutics using RNA aptamers provide unlimited possibilities due to their three-dimensional affinity and specificity to their targets (Dammes & Peer, 2020; Kher et al., 2011; Zhou et al., 2012).

ASOs are short single-stranded, synthetic RNA (or DNA) oligonucleotides (12–30 nt) (Fig. 1B), complementary through base pairing to their targets which can be mRNAs, pre-mRNAs, or ncRNAs (Quemener et al., 2020). Their mechanisms of action in the cell are diverse: ASOs induce arrest translation of the mRNA targets binding to the AUG start site of the ORF blocking the association of ribosomal subunits; or ASOs can bind to an upstream open reading frame (uORFs), increasing the translation of the main ORF which is located downstream of this uORF. ASOs also bind to splice sites, or to sequences in exons or introns which lead to ignore or include a particular exon, modifying the pre-mRNAs splicing. In the case of DNA ASOs, they can bind to the RNA target forming a DNA:RNA hybrid that leads to degradation by the RNase H mechanism (Baker et al., 1997; Bennett et al., 2019; Havens & Hastings, 2016; Rinaldi & Wood, 2018; Stenvang et al., 2012) (Fig. 2).

Endogenous miRNAs are essential in cell development and regulate gene expression by degradation and translation repression of a set of mRNAs by partial base–pair binding (Bajan & Hutvagner, 2020; Bartel, 2004). After cleavage by Dicer, a double-stranded miRNA is formed which is associated with AGO2 (miRISC) for subsequent binding and regulation of the target (Fig. 1C, left). Therapeutic **miRNAs** are able to mimic this double-stranded miRNA and can reestablish the level of a specific endogenous miRNA whose level drop has been caused by a disease (Bajan & Hutvagner, 2020; Fu et al., 2013; Lam et al., 2015; Jiang et al., 2018) (Fig. 2). Mimic miRNAs are synthetic RNAs containing the same sequence of endogenous miRNA and several of them are being tested in clinical trials (Chakraborty et al., 2021; Wahid et al., 2010; Zhang et al., 2021b).

siRNAs are short double-stranded RNA oligonucleotides (20–25 nt) that take advantage of the RNA interference (RNAi) pathway, a cellular defense mechanism (Fig. 1C, right). These RNAs are produced by the Dicer enzyme (endoribonuclease) from endogenous long dsRNAs and can knock down the expression of specific target genes with a complementary base pairing. Once cleaved, the double-stranded RNA, composed of two single strands, combines with the endonuclease Argonaute 2 (AGO2) in order to form the small interfering RNA-induced silencing complex (siRISC). Then the sense strand is released and the antisense strand remains in the RISC. Under the guidance of the antisense strand, RISC is activated and binds to the target mRNA by a fully base complementary pairing and leads to its degradation (Lam et al., 2015; Woolard & Chorley, 2019; Xu et al., 2018) (Fig. 2).

RNA aptamers are short single-stranded oligonucleotides that bind to their target in a shape-fitting manner, through this three-dimensional interaction (Fig. 1D). Compared with DNA aptamers, RNA aptamers fold into more varied structures, allowing to inhibit

several types of target molecules, including proteins, nucleotides, peptides, antibiotics, small molecules, and cells (Jayasena, 1999; Shigdar et al., 2011; Zhu et al., 2015). In the case of RNA targets, RNA aptamers are not necessarily complementary to the targets; they can interact following their tertiary and quaternary structures (Dammes & Peer, 2020; Kher et al., 2011; Zhou et al., 2012). Because of the immune-like resemblance of how aptamers bind to their targets, they are considered as "chemical antibodies" with inherent advantages, for example exclusive chemical synthesis, smaller in size, low variability among batches, better control in modifications after synthesis, and less immunogenicity (Bauer et al., 2019; Jayasena, 1999) (Fig. 2).

Manufacturing RNA Therapeutics

Manufacturing of IVT mRNAs

To make IVT mRNAs suitable as therapeutics, each structural element mentioned above has been optimized to modulate their stability, translation capacity, and immune-stimulatory profile of mRNA, especially the 5′ cap and the 3′ polyA tail. Natural eukaryotic mRNAs are 5′-capped with a 7–methylguanosine (m7G) by a 5′–5′–triphosphate bridge (ppp) forming an m7GpppN structure. In the case of IVT mRNAs, the cap can be added with two different approaches: the first one is the incorporation of the cap by a recombinant viral capping enzyme after the initial synthesis (Martin et al., 1975); the second one is adding a synthetic cap analog during the *in vitro* transcription reaction (Malone et al., 1989). Most of the ongoing clinical trials still use the latter or recently a variation of this approach: a cap incorporated in reverse orientation (antireverse cap analog; ARCA) with a more efficient translation (Jemielity et al., 2003; Montanaro et al., 2002; Sahin et al., 2014; Starostina et al., 2021; Stepinski et al., 2001; Ziemniak et al., 2013).

Chemically modified nucleotides are also used to replace natural nucleotides for the same purpose. Natural RNA is composed of four basic nucleotides: uridylate (U), adenylate (A), guanylate (G), and cytidylate (C). Karikó and collaborators found that replacing U with pseudouridine (Ψ) could reduce the innate immune responses against IVT mRNA (Karikó et al., 2008). Similarly, 5-methylcytidine (m5C) and N^1-methylpseudouridine (N1mΨ) were found to have the same functionality. However, there are some problems associated with these chemically modified nucleotides, for example, they cannot be precisely controlled in the reaction; and the exact mechanisms on regulating immune-stimulatory response of mRNA are still not fully understood.

The natural RNA in mammalian cells contains a polyA tail with a length of around 200 nt. The length of the polyA tail is gradually shortened throughout the lifespan of mRNA in cells, serving as a timer. The length of the polyA tail of IVT mRNA varies a lot, ideally ≥100 nt is preferred for mRNA therapeutics. Koski et al. showed that 150 nt polyA can lower the immune-stimulatory activities of synthetic mRNA (Koski et al., 2004). There are two methodologies to add a polyA tail. The first one is embedding the polyA sequence in the plasmid DNA template and the second involves enzymatic addition using recombinant polyA polymerases. The second way does not provide a fixed number of "A" but a wide distribution. Therefore, the first approach is preferred, even though it produces low yield of plasmid DNA since it contains a polyA sequence. Grier et al. developed a novel linear plasmid vector, which enables generation of mRNA with polyA tails up to 500 nt in length (Grier et al., 2016).

Synthetic mRNA can be produced via *in vitro* transcription. This method was first developed by Butler and Chamberlin in the 1980s (Butler & Chamberlin, 1982), then improved by Krieg and Melton (Krieg & Melton, 1987). Initially, 8 to 20 RNA molecules were generated by each DNA template, however, Gurevich et al. significantly increased the yield from 20 to 480 RNA molecules (Gurevich et al., 1991). Nowadays, it is typical to achieve a few grams of mRNA per liter by using commercial kits (Pardi et al., 2018).

Currently, IVT mRNA is manufactured by a cell-free system, which costs much less in comparison with recombinant proteins produced in eukaryotic cells. This system can be easily standardized to produce clinical-grade products under good manufacturing practice (GMP). The whole procedure relies on using a DNA template. The latter can be obtained either from plasmid DNA (pDNA) or by amplifying the gene of interest using polymerase chain reaction (PCR). pDNA can be produced by using *Escherichia coli* as a workhorse. For manufacturing mRNA therapeutics, high purity pDNA with very low level of endotoxin is necessary. Since plasmid DNA is a double-stranded circular DNA, it needs to be first linearized using restriction enzyme digestion and then it can be used as a DNA template. This will be *in vitro* transcribed into messenger RNA via an enzymatic reaction, typically using T7, SP6, or T3 RNA polymerase.

To use T7 RNA polymerase as an example, a successful IVT reaction requires six major components: (i) a linearized pDNA with a T7 promoter; (ii) nucleoside triphosphates (NTP) for four bases; (iii) ribonuclease inhibitor for inactivation of RNase; (iv) pyrophosphatase for degrading accumulated pyrophosphate; (v) magnesium ions as a cofactor for the T7 RNA polymerase; (vi) other factors such as pH of the buffer, optimal concentration of antioxidant and polyamine. The success of an IVT reaction in the bioreactor also relies on the incubation time, temperature, and the concentration of each individual component.

Recently mRNA vaccines have been applied to prevent COVID-19 spreading. To provide one billion doses of mRNA vaccine, a large amount of pDNA is required (around 1 kg of pDNA). Typically, the crude yield of cGMP-grade pDNA produced by *E. coli* fermentation is only around 200 to 500 mg/L, and with a long polyA tail, this yield can be even lower (less than 100 mg/L). Therefore, it is necessary to find alternative methods to generate high-yield pDNA. Rolling circle amplification (RCA) is a simpler and faster method, which holds the promise to replace traditional *E. coli* fermentation. Most importantly, it is cell-free and with no need for the traditional fermentation, harvesting, and purification steps.

After the reaction, the raw product needs to be further purified. The impurities are either process- or product-related, including but not limited to dsRNA, residue NTPs, enzymes, DNA template. The impurities are typically removed using a chromatographic column, the purified product can be concentrated and buffer-exchanged by tangential flow filtration (TFF). After that, the product will be filter-sterilized. Finally, the process will be completed with the formulation and packaging of the pure RNA drug substance.

Initial concerns of short half-life, unfavorable immunogenicity, and unrestrained expression of IVT mRNA are fading today, with the manufacturing of more stable and nonimmunogenic-modified nucleotides, and with a more refined regulation of mRNA expression (Dammes & Peer, 2020). Indeed, in order to solve the problem of IVT mRNAs stability in the host cell, a promising technology based on Circular RNAs (CircRNAs) is emerging, which stabilizes and consequently extends the production of their encoded peptides/proteins (Huang et al., 2018; Jeck & Sharpless, 2014; Zhang et al., 2013). CircRNAs are endogenous single-stranded RNAs whose 5′ and 3′ ends are covalently linked, and differently than linear RNAs, more resistant to exonucleolytic degradation (Houseley & Tollervey, 2009; Luz et al., 2007). Using this

technology, Wesselhoeft and collaborators were able to circularize a wide range of mRNAs by self-splicing with an autocatalytic intron (Kowalzik et al., 2021; Wesselhoeft et al., 2018, 2019).

Manufacturing of Oligonucleotide Therapeutics

Oligonucleotides can be made either by fragmentation of larger biomolecules or by targeted chemical synthesis. In the latter case, oligonucleotide synthesis is performed by a chemical process using nucleoside phosphoramidites, analog nucleotides that have their reactive groups protected (hydroxy groups in ribose or exocyclic amino groups in nucleic bases). The challenging part is to choose the protecting group for the 2′-hydroxyl function of ribose. As such, RNA oligonucleotides can be synthesized via solid-phase synthesis, similar to solid-phase synthesis of peptides, and kilogram level product can be achieved under cGMP.

The synthesis is performed in a flow-through column reactor with a pump-driven system. The first nucleoside is attached to the solid support, and then filled in the column reactor. Typically, four steps are involved to introduce the second nucleoside on top of the first one: (i) Detritylation: the 5′-dimethoxytrityl protecting group is removed from the support-bound nucleoside. (ii) Coupling: the appropriate phosphoramidite monomer (A, G, U, C) is coupled with the help of an activator. (iii) Thiolation/oxidation: the newly formed phosphite triester internucleotide bond is converted to phosphorothioate or phophodiester by thiolation or oxidation agents. (iv) Capping: the unreacted 5′hydroxyl groups are capped by capping agents. After these four steps, one cycle is completed and a new cycle will be started. The whole process will be repeated multiple times until the full-length oligonucleotide has been synthesized.

As it can be seen, even though each step has up to 99% yield; the purity of the final product will be only 70–80% depending on the length of the oligonucleoside. Therefore, downstream purification with HPLC is the main process step of the manufacturing of the small RNA molecules. Thus, much effort has to be devoted in this regard.

SiRNAs and mimic miRNAs can also be enzymatically synthesized by *in vitro* transcription with a subsequent DNase treatment and column purification (Leirdal & Sioud, 2002; Sioud & Leirdal, 2004). However, solid-phase synthesis commands currently the preference over this method (Dong et al., 2019; Marshall & Kaiser, 2004; Micura, 2002).

In the case of the manufacturing of RNA aptamers, a prior design is necessary. A widely used methodology to design aptamers is the Systematic Evolution of Ligands by Exponential Enrichment (SELEX, (Ellington & Szostak, 1990; Tuerk & Gold, 1990)). This technology was created in 1990 and helps to select aptamers that bind molecules with high affinity and specificity (Dammes & Peer, 2020; Kaur et al., 2018; Kher et al., 2011; Zhou et al., 2012). Isolation of selective single-stranded RNA from a library through repeated rounds of exposure, binding, selection, and amplification. Unlike DNA aptamers, RNA aptamers have better diversity in the fold due to the 2′-OH group. The procedure is as follows: first, a library of single-stranded RNAs with a high diversity of random sequences is synthesized by *in vitro* transcription. After a time of incubation with the target, samples are washed to remove the unspecific-bound molecules; the RNA sequences bound to the target are eluted and collected by centrifugation. The recovered pool is reverse transcribed, amplified by PCR and transcribed to RNA, and exposed to the target again, this process is repeated 5 to 15 times to isolate the more specific aptamers (Gold et al., 2012; Jayasena, 1999). Once the desired sequence of the RNA aptamers is obtained, the RNA aptamers are synthesized following the manufacture procedure described above.

Delivery

In order to achieve therapeutic effects, RNA-based drugs must overcome several barriers including nuclease degradation, short half-life, and recognition by the immune system (Damase et al., 2021; Kher et al., 2011; Kim, 2020). Because of the ionic nature and size of RNA therapeutics, diffusion across the cell membrane is only possible with a suitable delivery system, in order to facilitate their entry. Although chemical modification of nucleotides can increase nuclease resistance and avoid recognition by the immune system, other issues still need to be solved. As such, suitable drug delivery systems are applied to solve these largely persistent issues of stability, integrity, transport kinetics, targeting, improving the safety and efficacy of RNA therapeutics.

For therapeutics to treat cancer and other life-threatening disorders or to serve as vaccines to prevent infectious diseases, RNA needs to be delivered to the targeted cells, tissue(s), or organ(s). Literature has demonstrated that the efficiency of biomaterial-assisted nonviral delivery of RNA is much higher in comparison with naked RNA (Khalil et al., 2020). This particulate delivery system serves multiple purposes: (i) to protect RNA from degradation; (ii) to help delivery to the targeted site; (iii) to improve local uptake; and (iv) to serve as adjuvant in the case of mRNA vaccines. The main barrier for RNA delivery system to overcome is the plasma or endosomal lipid membrane; consequently, several types of biomaterials were used for this purpose; including lipids, polymers, and peptides.

Lipid nanoparticles (LNPs) is the most commonly used vehicle for RNA delivery (Fig. 3A). In 2018, Onpattro® received U.S. FDA approval, being an LNP-formulated siRNA drug (Akinc et al., 2019). In late 2020, two mRNA vaccines achieved FDA and EMA approval to prevent the spreading of COVID-19 and both vaccines are formulated with LNPs (Khurana et al., 2021). To briefly go over the evolving history of lipid-based RNA delivery system, cationic lipids were used initially to form lipoplex with negatively charged RNA. Because of the size, cytotoxicity issues, cationic lipids were replaced with ionizable lipids, which are pH-dependent. These lipids are not charged at pH 7, but positively charged under acidic conditions. Later on, biodegradable lipids were used, for example ester-based degradable lipids such as L319 and YSK12-C4 (Samaridou et al., 2020). LNPs contain structured lipids such as phospholipid and cholesterol, and PEG-lipid. PEG is a commonly used biomaterial as it has an excellent blood compatible profile. The reasons for the use of LNPs in mRNA vaccines include their efficiency, nontoxicity, and high selectivity.

Besides lipids, polymers are used as nonviral drug delivery vehicles for mRNA (Fig. 3B): positively charged polymers, such as PEI (polyethylenimine), PLL (poly L-Lysine), and poly(amidoamine) (PAMAM). These polymers are highly efficient but cytotoxic. In order to reduce their cytotoxicity, PEG is introduced to the delivery systems. Another major category of polymers for mRNA delivery is biodegradable polymers. Examples include poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), and poly(beta-amino-esters). Finally yet importantly, natural polymers, such as dextran and chitosan are also used to deliver mRNA.

Peptide-based biomaterials are also explored to deliver mRNA (Fig. 3C). Protamine is commonly used to formulate mRNA and cell-penetrating peptides (CPPs) are other commonly used peptides for gene delivery.

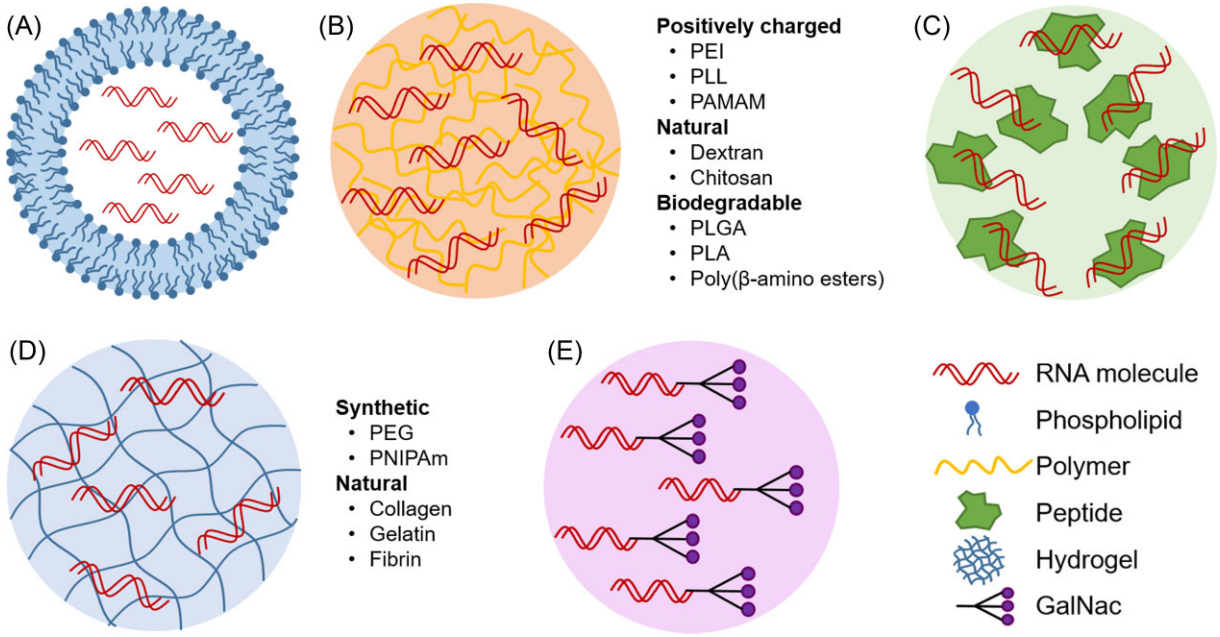

Fig. 3 Strategies to enhance RNA therapeutics delivery. (A) Lipid nanoparticles (LNP). (B) Polymers. (C) Peptides. (D) Hydrogels. (E) GalNac conjugation. GalNac, N-Acetylgalactosamine; PEI, polyethylenimine; PLL, poly L-Lysine; PAMAM, poly(amidoamine); PEG, polyethylene glycol; PNIPam, poly(N-isopropylacrylamide).

Hydrogels are water-soluble polymers self-assembled into a three-dimensional network. They are commonly used as nonviral gene delivery vehicles, especially for local delivery and in a controlled release manner (Fig. 3D). Both natural and synthetic materials have been studied as hydrogels to locally deliver RNA molecules, including siRNA, miRNA, and mRNA (Carballo-Pedrares et al., 2020). The applications of hydrogel-based RNA delivery are different from those of lipid-, polymer-, and peptide-based RNA delivery, mainly for tissue engineering and regenerative medicine. Examples include bone, cartilage, neuronal and skin tissue engineering. The natural hydrogel materials used as RNA delivery vehicles include collagen, gelatin, and fibrin. The synthetic hydrogel materials for the same purpose include PEG and poly(N-isopropylacrylamide) (PNIPAm).

GalNac is the ligand of the asialoglycoprotein receptor (ASGPR) (Fig. 3E). ASGPR is an endocytic receptor that is highly specifically expressed on the membrane surface of hepatocytes with almost no expression on other types of cell. A synthetic triantennary N-acetylgalactosamine-based ligand is conjugated to chemically modified siRNA, forming GalNac–siRNA conjugate. It can be efficiently delivered to hepatocytes via the ASGPR-mediated pathway. The GalNac system has been successfully used to deliver three siRNA drugs (Table 2). Clinically relevant delivery systems for RNAi such as siRNA constructs have been developed with variable success, including liposomal, peptide-based, and polymeric systems (Ward et al., 2021). As with other RNA-based therapeutics, cationic delivery systems have an advantage because of their ready complexation with the anionic oligonucleotides. The administration route of IVT mRNA also plays an important role. Several routes were used to deliver mRNA, including intravenous, intradermal, intraventricular, intranasal, intrathecal, intramuscular, and more (Kowalski et al., 2019). Gene gun and other devices were also used to help deliver either naked mRNA or mRNA complexed with biomaterials (Deering et al., 2014). For COVID-19, mRNA vaccines are administered via an intramuscular route.

Therapeutic Applications

The future of RNA-based therapeutics raises vast expectations and the opportunity to reach an era of personalized and accessible medicine that treats common disorders and many rare diseases seems to be closer and closer. Huge investment and the exponential development in RNA therapeutics during the recent years demonstrate this, although some objectives are nearer than others.

Some of the advantages accompanying the use of RNA therapeutics include: simplicity and agility in development and manufacturing processes; a broader spectrum of target molecules, paving the way for new therapeutic pathways; and no risk of permanent changes in the patient's genome. Furthermore, thanks to the constant work of researchers around the world, some barriers that delayed the development of these technologies such as RNA stability, premature degradation, target delivery, immunogenicity, and development costs are being overcome. In this section, we will describe the most relevant clinical trials in RNA therapeutics and the RNA-based drugs approved by the FDA.

FDA-Approved Therapeutics

To date, the FDA has approved 14 RNA-based therapeutics shown in Table 2. The majority of licensed therapeutics are of the ASO type (eight), followed by siRNA (three), mRNA (two), and aptamer (one). The pioneer RNA-based drugs Fomivirsen (1998), Pegaptanib (2004), and Mipomersen (2013) are no longer on the market mainly because of the development of more effective or accessible treatments for their target disease (Stein & Castanotto, 2017). In 2016, the FDA approved Nusinersen, the first drug to treat spinal muscular atrophy (SMA). Nusinersen is an ASO RNA-based therapy that increases levels of the survival motor neuron (SMN) protein (Hoy, 2017). At present, there are two new approved treatments for SMA: Zolgensma (2019), an adeno-associated virus vector-based gene therapy, and Risdiplam (2020), a survival of motor neuron 2-directed RNA-splicing modifier (FDA, 2019, 2020). However, there

Table 2. RNA Therapies Approved by the U.S. Food and Drug Administration (FDA).

Drug	Trade name	Target disease	Type of RNA	Target molecule	Route of administration	Delivery system	FDA Approval	Marketing Status	Reference
Fomivirsen	Vitravene	Cytomegalovirus (CMV) retinitis	ASO	IE2 mRNA	Intravitreal injection	NA	August 1998	Discontinued	(FDA, 2021b; Grillone & Lieberman, 2017)
Pegaptanib	Macugen	Exudative age-related macular degeneration (AMG)	Aptamer	VEGF protein	Intravitreal injection	NA	December 2004	Discontinued	(FDA, 2021b; Schwartz & Amoaku, 2005)
Mipomersen	Kynamro	Homozygous familial hypercholesterolemia	ASO	ApoB-100 mRNA	Subcutaneous	NA	January 2013	Discontinued	(FDA, 2021b; Hair et al., 2013)
Eteplirsen	Exondys 51	Duchenne muscular dystrophy (DMD)	ASO	Dystrophin pre-mRNA	Intravenous	NA	September 2016	Prescription	(FDA, 2021b; Syed, 2016)
Nusinersen	Spinraza	Spinal muscular atrophy	ASO	SMN2 pre-mRNA	Intrathecal	NA	December 2016	Prescription	(FDA, 2021b; Hoy, 2017)
Patisiran	Onpattro	Polyneuropathy caused by hereditary transthyretin amyloidosis (hATTR)	siRNA	Transthyretin mRNA	Intravenous	LNP	August 2018	Prescription	(FDA, 2021b; Hoy, 2018)
Inotersen	Tegsedi	Polyneuropathy caused by hATTR	ASO	Transthyretin mRNA	Subcutaneous	NA	October 2018	Prescription	(FDA, 2021b; Keam, 2018)
Givosiran	Givlaari	Acute hepatic porphyria	siRNA	ALAS1 mRNA	Subcutaneous	GalNac	November 2019	Prescription	(FDA, 2021b; Scott, 2020)
Golodirsen	Vyondys 53	DMD	ASO	Dystrophin mRNA	Intravenous	NA	December 2019	Prescription	(FDA, 2021b; Heo, 2020)
Viltolarsen	Viltepso	DMD	ASO	Exon 53 of the dystrophin mRNA precursor	Intravenous	NA	August 2020	Prescription	(Dhillon, 2020; FDA, 2021b)
Lumasiran	Oxlumo	Primary hyperoxaluria type 1 (PH1)	siRNA	Hydroxyacid oxidase 1 (HAO1) mRNA	Subcutaneous	GalNac	November 2020	Prescription	(FDA, 2021b; Scott & Keam, 2021)
BNT162b2	Comirnaty	COVID-19	mRNA	-	Intramuscular	LNP	December 2020[a]	-	(FDA, 2021a; Lamb, 2021a)
mRNA-1273	Moderna COVID-19	COVID-19	mRNA		Intramuscular	LNP	December 2020[a]	-	(Baden et al., 2021; FDA, 2021a)
Casimersen	Amondys 45	DMD	ASO	DMD gene pre-mRNA	Intravenous	NA	February 2021	Prescription	(FDA, 2021b; Shirley, 2021)

[a]Emergency Use Authorization (EUA).

are considerable variations in the accessibility, price, and administration of these treatments for SMA. Therefore, their future in the market will depend on how pharmaceutical companies address the current situation of these patients.

On the other hand, Duchenne muscular dystrophy (DMD) disorder has four approved RNA-based therapies that are Eteplirsen (2016), Golodirsen (2019), Viltolarsen (2020), and Casimirsen (2021). These therapies follow an exon-skipping approach where the ASOs avoid the expression of the mutated portion of the gene resulting in a functional protein (Dhillon, 2020; Heo, 2020; Shirley, 2021; Syed, 2016). Each therapy treats disease-specific mutations of DMD. Furthermore, in 2018, Inotersen (ASO) and Patisiran (siRNA) were approved for the treatment of polyneuropathy caused by hereditary transthyretin amyloidosis (hATTR) (Hoy, 2018; Keam, 2018). The latter is the first siRNA-based therapy approved by the FDA, followed by Givosiran (2019) and Lumasiran (2020) for the treatment of acute hepatic porphyria and primary hyperoxaluria type 1 (PH1), respectively (Scott, 2020; Scott & Keam, 2021).

In late 2020, the mRNA-based vaccines BNT162b2 and mRNA-1273, developed in record time against some variants of COVID-19, were granted Emergency Use Authorizations (EUAs) (FDA, 2021a). On the other hand, there are RNA-based drugs whose approval was denied by the FDA but granted by other regulatory agencies such as the European Medicines Agency (EMA). For example, inclisiran (Leqvio) and volanesorsen (Waylivra) for the treatment of primary hypercholesterolaemia and familial chylomicronemia syndrome (FCS), respectively are authorized for use in the European Union (Lamb, 2021b; Paik & Duggan, 2019).

RNA-Based Therapeutics in Advanced Clinical Trials

The use of mRNA as a therapy tool has been under study for years, with a growing interest in vaccines for infectious diseases and certain types of cancer. One of the main advantages of using mRNA technologies is the opportunity for rapid development and manufacturing against emerging pathogens, as demonstrated with the SARS-CoV-2 vaccines. Currently, the only authorized mRNA-based therapies are the BNT162b2 (Comirnaty) and mRNA-1273 (Moderna) vaccines for the prevention of COVID-19 (FDA, 2021a). Moreover, the companies CureVac and Walvax reached phase III of clinical trials of their COVID-19 vaccines (NCT04674189 and NCT04847102, respectively). On the other hand, there are IVT mRNA therapies in initial phases of clinical trials for several carcinomas such as advanced melanomas (NCT01684241, NCT02410733, NCT03897881, and NCT04526899), solid tumors (NCT04486378, NCT03739931, and NCT03313778), nonsmall lung cancer (NCT03164772), as well as the treatment of different viral infections including cytomegalovirus infection (NCT04232280, NCT03382405), Zika virus (NCT04917861, NCT04064905), influenza (NCT03345043), rabies (NCT03713086), and Chikungunya virus infection (NCT03829384).

ASOs have the more RNA-based therapies approved by the FDA to date. In addition, ASOs have been studied to treat different eye disorders, being QR-110 (seporfasen), a splice-modulating oligonucleotide, one of the most advanced (phase III; NCT04855045 and NCT03913143). QR-110 is currently investigated in clinical trials for use against Leber's congenital amaurosis (LCA), a severe type of inherited retinal dystrophy (Dulla et al., 2018). In addition, the QR-421a and QR-1123 formulations for the treatment of retinitis pigmentosa are in phase I/II of clinical trials (NCT03780257 and NCT04123626, respectively). The use of ASOs has also been considered for the treatment of several hematological diseases and some types of cancer such as urologic cancer, nonsmall cell lung cancer, prostate, leukemia, and different lymphomas as presented by Giudice et al., (2020) and Xiong et al. (2021). Oblimersen was tested in more than 40 clinical trials for various types of cancer, such as chronic lymphocytic leukemia (CLL), B-cell lymphoma, lung, and breast cancer (Xiong et al., 2021). The most promising are those in which oblimersen is combined with other chemotherapeutic drugs (Giudice et al., 2020; Hu et al., 2019; Xiong et al., 2021).

There are three FDA-approved siRNA drugs: patisiran, givosiran, and lumasiran. In late 2020, the drug inclisiran failed to win FDA approval due to unresolved facility inspection-related conditions (Novartis Media Relations, 2020). However, the developers are working on resolving nonconformities for the drug to be approved in the United States (Novartis Media Relations, 2020). The siRNA-based therapies in the early stages of clinical trials aim to treat conditions such as eye-related disorders, scarring, solid tumors, or hepatitis B, and diverse types of cancer including pancreatic, prostatic, liver, lung (Saw & Song, 2020; Xiong et al., 2021). On the other hand, most studies in an advanced stage of clinical trials (phase III) involve rare or orphan diseases. Therefore, their requirement may influence their approval speed. The six siRNA-based therapeutics in phase III trials include vutrisiran (NCT03759379 and NCT04153149) for the treatment of transthyretin-mediated (ATTR) amyloidosis, nedosiran for primary hyperoxaluria (NCT04042402), fitusiran for hemophilia A and B (NCT03974113, NCT03754790, and NCT03417245), teprasiran for acute kidney injury (AKI) after transplant or cardiovascular surgery (NCT03510897), cosdosiran for nonarteritic anterior ischemic optic neuropathy and primary angle glaucoma (NCT02341560), and tivanisiran for the treatment of ocular pain and dry eye disease (NCT04819269) (Zhang et al., 2021a).

Multiple biopharmaceutical companies and research institutions invest their resources in developing miRNA-based products that can be patented, with thousands of patents being filed in both the United States and Europe today (Chakraborty et al., 2021). According to Chakraborty and collaborators, most miRNAs patents are related to cancer, metabolic disorders, and inflammatory disorders. Nevertheless, despite the interest and investment in miRNAs therapies, there is not yet any miRNA-based therapy approved by the FDA or in an advanced phase of clinical trials, only in the preclinical or initial stages (Zhang et al., 2021a). Examples include cobomarsen (MRG-106) for the treatment of cutaneous T-cell lymphoma (CTCL), mycosis fungoides (MF) subtype (NCT03713320); and remlarsen (MRG-201), an miR-mimic RNA therapy to treat keloids (NCT03601052). However, given that the development of miRNA therapies seems to be slower compared to those of siRNA, Zhang and collaborators explore the possible reasons for this delay from a drug target perspective in greater depth (Zhang et al., 2021a).

Although the only FDA-approved aptamer RNA, Pegaptanib, was discontinued from the market, research on this type of therapy has not stopped. Zimura is a candidate to treat eye disorders such as macular degeneration and geographic atrophy (phase III; NCT04435366 and NCT02686658) and Stargardt disease 1 (phase II; NCT03364153). Moreover, olaptesed pegol (NOX-A12) is being investigated to treat different cancers such as glioblastoma (NCT04121455), chronic lymphocytic leukemia (CLL; NCT01486797), colorectal and pancreatic cancers (NCT03168139 and NCT04901741). The drug NOX-H94 reached phase I in clinical trials to treat anemia of chronic disease (inflammation;

NCT01372137) and BT200 is tested to treat hereditary bleeding disorders such as Von Willebrand diseases and hemophilia A (NCT04677803). Overall, RNA aptamers show significant promise in affinity-based therapy and diagnostics, potentially replacing antibodies (Kaur et al., 2018; Shigdar et al., 2011).

Conclusions and Future Directions

RNA therapeutics have been developed substantially since the first antisense RNA drug was approved by the FDA in 1998. This early recognized potential is being rapidly fulfilled due to the key advantages of RNA therapeutics, which include:

- Their capacity to act on disease targets that are unattainable for either large macromolecules (proteins such as cytokines or antibodies) or small molecules;
- The inherent ease and speed of generating new constructs for personalized medicine and for effectively combatting new threats such as constantly evolving viral pathogens like the novel SARS-CoV-2 virus;
- The extensive possibilities of rapidly introducing new and desirable features by highly specific structural modification at multiple levels (basic nucleotidic backbone, sugar chemistry, tertiary structure) of the RNA therapeutic molecule;
- The considerably better economics of designing, generating, purifying, formulating, and manufacturing at industrial scale RNA drugs compared to either recombinant proteins or small molecules;
- The relatively simple, robust, and versatile set of manufacturing platforms ranging from batch bioprocessing of plasmid DNA and enzymatic cell-free synthesis to entirely chemical synthesis;
- The better safety profile of many RNA drugs, including mRNA-based vaccines and cell therapies, and, consequently, the simpler regulatory hurdles for their approval.

Significant strides in basic understanding of RNA biology and astute exploitation of the constantly evolving wealth and variety of RNA molecules coupled with advances in nanotechnology and bioinformatics have led to the current disruptive technology of RNA drugs with already exciting outcomes and even more powerful benefits expected in the near- and medium-term horizon. The many ongoing preclinical studies and approved clinical trials of personalized therapeutic RNA vaccines are just one example of immunotherapy against a variety of cancers, including metastatic solid tumors. Similarly, the future is bright for individualized cell therapies based on the *ex vivo* modification of the patient's own cells with mRNA of the appropriate proteins and the reintroduction of the reprogrammed cells into the patient. Such a scheme holds considerable promise for the transient formation of target proteins under repeated administration of cell aliquots but it can also function as a gene-editing approach to establish the stable expression of such proteins. In addition, gene silencing as a result of RNAi is a concept with high clinical relevance which will be applied to an ever-wider range of diseases. Key aspects of molecular recognition inherent in RNA aptamers but also in several other types of RNA molecules discussed in this review are poised to expand their applications beyond the narrow definition of therapy as disease control and prevention, that is in diagnostics and imaging among others.

On the other hand, continued basic research into the human "RNA-ome" is bound to reveal hitherto unknown functions of many short ncRNA molecules in different organs and in different individuals in health and disease, thus facilitating the development of precision medicine approaches and designing improved and personalized diagnostics and therapeutics (Rigoutsos et al., 2019).

The power and versatility of the RNA-based constructs in themselves are complemented and reinforced by the immense possibilities offered by current (nano)materials technologies for the conception of novel delivery vehicles. These carriers are designed to protect the therapeutic molecules during their transport from key challenges such as degradation by RNases, rapid renal clearance from the body and unwanted immune responses, while at the same time facilitating the transport as well as the targeting of the RNA therapeutic by promoting its specificity and cellular uptake. These challenges and the incremental successes in meeting them within experimental and, especially, clinical settings, are pointing the way for future advances in efficient and safe delivery systems. Innovative nanoscopic and, primarily, cationic carriers will need to be endowed with molecular structures enabling high selectivity in the transport and delivery of RNA cargos. At the same time, there is a persistent need for low-toxicity cationic carriers. The controlled biodegradability over precise time spans is another desired property of the new-generation carriers that will need to be cleared from the body, while at the same time they would afford the RNA payload an acceptable level of stability during the period of its therapeutic action. Innovation in carriers for RNA drug delivery is also expected in biomimetic design to which lipid bilayer-based nanoparticles lend themselves particularly well. In this concept, functionalized membrane proteins decorating the surface of liposomes allow biochemical recognition at specific cellular sites aided by adhesion molecules. This emerging biomimetic platform is showing promise in applications requiring endothelial antiinflammatory activity (Molinaro et al., 2016) that could be extended to the development of biomimetic nanoparticles with new mRNA therapeutics to selectively target a wide range of inflammation conditions.

Finally, from a strategic point of view, the very fast and cost-effective generation of high-purity RNA constructs with infinite possibilities of design modifications compared to the development of recombinant protein or small molecule pharmaceuticals is propelling RNA drugs as center-stage contenders in pharmaceutical biotechnology for disease control and prevention. This means that novel RNA drugs are not just the privileged realm of corporate pharmaceutical giants but are entirely within the reach of even small biotech startups and academic/clinical research groups as well as hospital settings (Damase et al., 2021). Hospital-based RNA therapeutics are already proving the tangible success of such an ecosystem that can quickly achieve clinical translation thanks to innovative ideas and key complementary competencies in-house and in the vicinity. Interdisciplinary collaboration among basic bioscientists, clinicians, engineers, bioinformatics experts, nanotechnology scientists and regulatory affairs experts, among others, can assure accelerated development of a wealth of novel RNA drug formulations with special emphasis on personalized therapy.

As this manuscript was being finalized, the FDA has changed the status of the drug Comirnaty (BNT162b2), vaccine for the prevention of COVID-19 disease, from Emergency Use Authorization to full approval (https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine). Another evidence that the development of RNA therapeutics progresses rapidly and challenges us to keep up with its pace.

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Conflict of Interest

The authors declare no conflict of interest.

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