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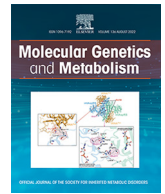
Review article

RNA solutions to treat inborn errors of metabolism

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ARTICLE INFO

ABSTRACT

RNA-based therapies are a new, rapidly growing class of drugs that until a few years ago were being used mainly in research in rare diseases. However, the clinical efficacy of recently approved oligonucleotide drugs and the massive success of COVID-19 RNA vaccines has boosted the interest in this type of molecules of both scientists and industry, as wells as of the lay public. RNA drugs are easy to design and cost effective, with greatly improved pharmacokinetic properties thanks to progress in oligonucleotide chemistry over the years. Depending on the type of strategy employed, RNA therapies offer the versatility to replace, supplement, correct, suppress, or eliminate the expression of a targeted gene. Currently, there are more than a dozen RNA-based drugs approved for clinical use, including some for specific inborn errors of metabolism (IEM), and many other in different stages of development. New initiatives in n-of-1 RNA drug development offer new hope for patients with rare diseases and/or ultra-rare mutations. RNA-based therapeutics include antisense oligonucleotides, aptamers, small interfering RNAs, small activating RNAs, microRNAs, lncRNAs and messenger RNAs. Further research and collaborations in the fields of chemistry, biology and medicine will help to overcome major challenges in their delivery to target tissues. Herein, we review the mechanism of action of the different therapeutic approaches using RNA drugs, focusing on those approved or in clinical trials to treat IEM.

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Contents

1. Introduction

RNA-based therapeutics have revolutionized the drug industry and expanded treatment options for diseases lacking effective treatments [1]. Traditionally, protein-targeting small molecules, antibodies and recombinant proteins have dominated the therapeutic landscape. RNA drugs offer the possibility of addressing potentially any disease-causing gene expression defect at the post-transcriptional or translational level, thus effectively increasing the "druggable" space. Furthermore, RNA drugs can be easily designed, chemically synthesized, rapidly adapted for personalized treatments or for targeting an evolving pathogen, and have been shown to result in minimal adverse secondary effects. Initial hurdles to their clinical application included inherent instability and poor nuclease resistance, short biological half-life, immune response and inefficient organ/tissue delivery and cellular uptake. Many of these challenges have been mainly overcome thanks to the interest and advancements of a broad scientific community performing continued basic and translational research for more than 30 years, although there is still room for improvement [2].

Currently, antisense oligonucleotides (ASO), small interfering RNA (siRNA), small activating RNAs (saRNA), microRNAs (miRNAs), aptamers, long non-coding RNAs (lncRNAs) and messenger RNA (mRNA) are all included in the RNA therapeutics category, with different mechanisms of action [3] (Fig. 1). Chemical modifications and substitutions affecting the nucleotide bases and the phosphate-sugar backbone have improved their stability and pharmacokinetic properties, while the use of several delivery approaches such as conjugates and nanoparticles has made possible many therapeutic indications [4]. More than a dozen molecules have now received marketing authorization from the Food and Drug Administration (FDA) in the United States of America, European Medicines Agency (EMA) in Europe and/or the Japan Ministry of Health, Labor and Welfare (Table 1), and many others are in ongoing clinical trials [5–7]. Several reviews have recently appeared on the topic of RNA therapies, stage of development and options for delivery [4,6,8–13].

RNA drugs can be single or double-stranded, with sizes ranging from 20 nucleotides to more than 1000 (from ~5 kDa up to 10,000 kDa) and their design and chemistry dictates the mechanism of action, that broadly includes gene silencing (siRNA, ASO, miRNAs), functional protein production or upregulation (ASO, mRNA, saRNA) or binding to proteins and other molecules in the case of aptamers or lncRNA [8] (Fig. 1).

Below we describe each type of RNA drugs, along with relevant examples of their clinical applications, and discuss the associated current challenges. In inborn errors of metabolism (IEM), rare genetic diseases in which there is a pathological alteration in a biochemical pathway generally caused by an enzyme deficiency [14], RNA therapies are also coming of age, with siRNAs, ASOs and mRNAs in clinical or preclinical research, approved drugs for primary hyperoxaluria type 1 (PH1, OMIM #259900) and acute hepatic porphyria (AHP, OMIM #612740), and future research using different approaches envisaged for many diseases, including the recently discovered potential use of a lncRNA for phenylketonuria (PKU, OMIM #261600) [15].
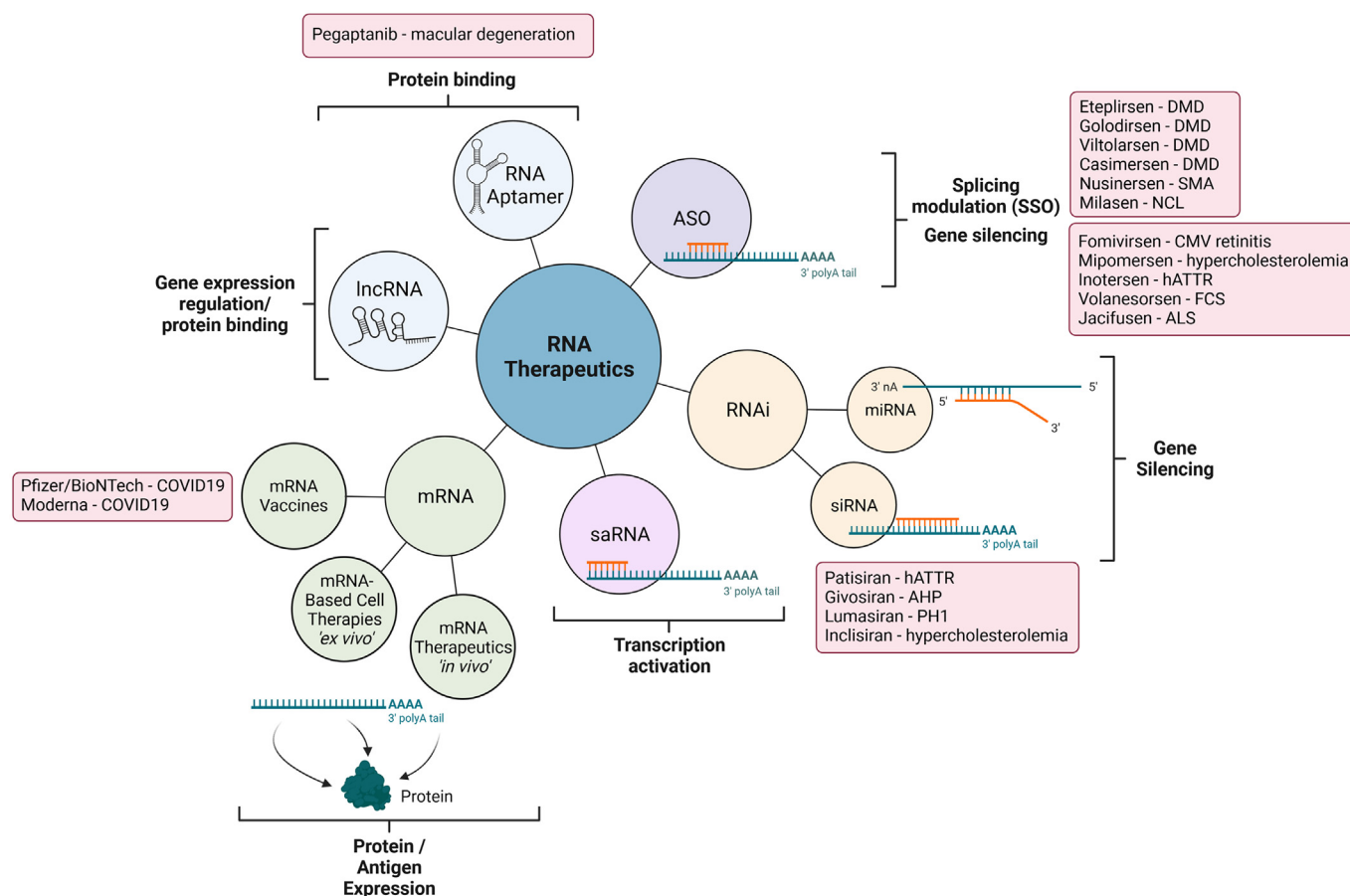
Fig. 1. Outline of the different classes of RNA therapeutics. Boxes show approved drugs and diseases targeted for each type of RNA therapy. RNA, ribonucleic acid; ASO, antisense oligonucleotide; SSO, splice switching oligonucleotide; A, adenosine molecule; RNAi, RNA interference; miRNA, microRNA; saRNA, small activating RNA; siRNA, small interfering RNA; mRNA, messenger RNA; lncRNA, long non-coding RNA; DMD, Duchenne muscular dystrophy; SMA, spinal muscular atrophy; NCL, neuronal ceroid lipofuscinosis; CMV, cytomegalovirus; hATTR, hereditary amyloidosis transthyretin related; FCS, familial chylomicronemia syndrome; ALS, amyotrophic lateral sclerosis; AHP, acute hepatic porphyria; PH1, primary hyperoxaluria type 1. Figure generated using BioRender.com.

Table 1
RNA therapeutics approved for clinical use by May 2022.

Drug	Type	Indication	Target	Chemistry	Delivery/target organ	FDA approval date	Reference
Fomivirsen (Vitravene)	RNAse H1 ASO	CMV retinitis	CMV UL123	PS DNA	ITV/eye	1998	[59]
Mipomersen (Kynamro)	RNAse H1 ASO	hypercholesterolemia	ApoB-100	PS 2'-MOE	SC/liver	2003	[60]
Inotersen (Tegsedi)	RNAse H1 ASO	hATTR	transthyretin	PS 2'-MOE	SC/liver	2018	[61]
Volanesorsen (Waylivra)	RNAse H1 ASO	FCS	Apo-CIII	PS 2'-MOE	SC/liver	2019 (EMA)	[62]
Pegaptanib (Macugen)	aptamer (PEG)	macular degeneration	VEGF265	2'-OMe modified	ITV/eye	2014	[63]
Patisiran (Onpattro)	siRNA (LNP)	hATTR	transthyretin	2'-OMe modified	IV/liver	2018	[64]
Givosiran (Givlaari)	siRNA-GalNac	AHP	ALAS1	2'-OMe modified	SC/liver	2019	[25]
Lumasiran (Oxlumo)	siRNA-GalNac	PH1	GO	2'-OMe modified	SC/liver	2020	[24]
Inclisiran (Leqvio)	siRNA-GalNac	hypercholesterolemia	PCSK9	2'-OMe modified	SC/liver	2021	[65]
Eteplirsen (Exondys 51)	SSO	DMD	DMD exon 51	PMO	IV/muscle	2016	[66]
Golodirsen (Vyondys 53)	SSO	DMD	DMD exon 53	PMO	IV/muscle	2019	[67]
Viltolarsen (Viltepso)	SSO	DMD	DMD exon 53	PMO	IV/muscle	2020	[68]
Casimersen (Amondys 45)	SSO	DMD	DMD exon 45	PMO	IV/muscle	2021	[69]
Nusinersen (Spinraza)	SSO	SMA	SMN2 exon 7	PS-2'-MOE	IT/CNS	2016	[70]
Milasen	SSO	NCL7	MFSD8	PS-2'-MOE	IT/CNS	2019 ($n = 1$ trial)	[38]
Jacifusen	RNAse H1 ASO	ALS	FUS	PS 2'-MOE	IT/CNS	2019 ($n = 1$ trial)	[56]

1.1. mRNA therapies

The idea of introducing mRNA into cells, that will then translate it to produce the corresponding protein and thus serve as treatment for diseases caused by protein dysfunction, or serve to elicit an immune response to prevent disease or fight cancer (Fig. 1), is quite simple. The first demonstration of the potential of mRNA to be used as a drug was in 1990, when different proteins were shown to be expressed after injecting mRNA into mouse skeletal muscle [16]. Within a few years, the approach was proven to have therapeutic potential in a rodent model of diabetes insipidus [17]. However, during the successful development and utilization of the mRNA COVID19 vaccines, many people believed that this was a new technology coming out of nowhere in less than a year. Actually, basic and translational research in mRNA applications had been ongoing for decades, with many of the initial drawbacks already overcome [18]. To date, there are several therapeutic modalities that use mRNA: i) what is known as replacement therapy, where administered mRNA codes for a defective, missing or a therapeutic protein; ii) for *ex vivo* cell therapy, where mRNA is transfected into cells to modify their phenotype or function (cancer immunotherapy) and iii) vaccination, where mRNA codes for specific antigen(s).

mRNA as replacement therapy holds great promise for genetic diseases, as an alternative to DNA-based gene therapy or recombinant protein administration, more expensive and laborious to manufacture. One advantage is that mRNA does not require delivery into the nucleus, thus reducing the risk of integration into the host genome, although mRNA's effects are temporary, which requires regular re-administration. These two facts actually reduce the concern in long-term safety issues, which may help accelerate approval by regulating agencies. Furthermore, the massive success in mRNA COVID19 vaccines will clearly impact in the public acceptance of therapeutic uses of mRNA.

Two main initial challenges in the clinical use of mRNA, immunogenicity and stability of the RNA molecule, have been solved by use of different modifications in its structural components: 5'-Cap, 3'-UTR and poly(A) tail have been optimized, non-standard nucleosides are currently used, and codon optimisation is introduced to increase translatability. The other essential component of mRNA therapy is the delivery agent, the lipid nanoparticle (LNP) that can deliver the mRNA into the cytoplasm [19]. Each pharmaceutical company has its own proprietary LNP and targeted delivery has been efficiently developed for liver, lung and heart.

Different companies have produced mRNA candidates for IEM and some are currently being tested in clinical trials. Applications focused in the liver include mRNA therapy for organic acidemias (methylmalonic aciduria, MMA: OMIM #251000 and propionic acidemia, PA: #606054) (Fig. 2A), AHP, ornithine transcarbamylase

(OTC) deficiency (OMIM: #311250) and PKU, recently reviewed in [19]. In each case, mRNA of the defective gene is delivered with LNP systemically, and, in the case of PA, both the *PCCA* and *PCCB* mRNAs are delivered together, to ensure correct restoration of the propionyl-CoA carboxylase (PCC) enzyme complex, as it was demonstrated that this strategy results in higher PCC activity than using single (*PCCA* or *PCCB*) mRNA alone [20]. Other applications include the research in systemically administered mRNA encoding human α-Gal A for treating Fabry disease (OMIM #301500) [21], while delivery to the lung is exemplified by the development of an mRNA therapy for cystic fibrosis (OMIM # 219700) [8].
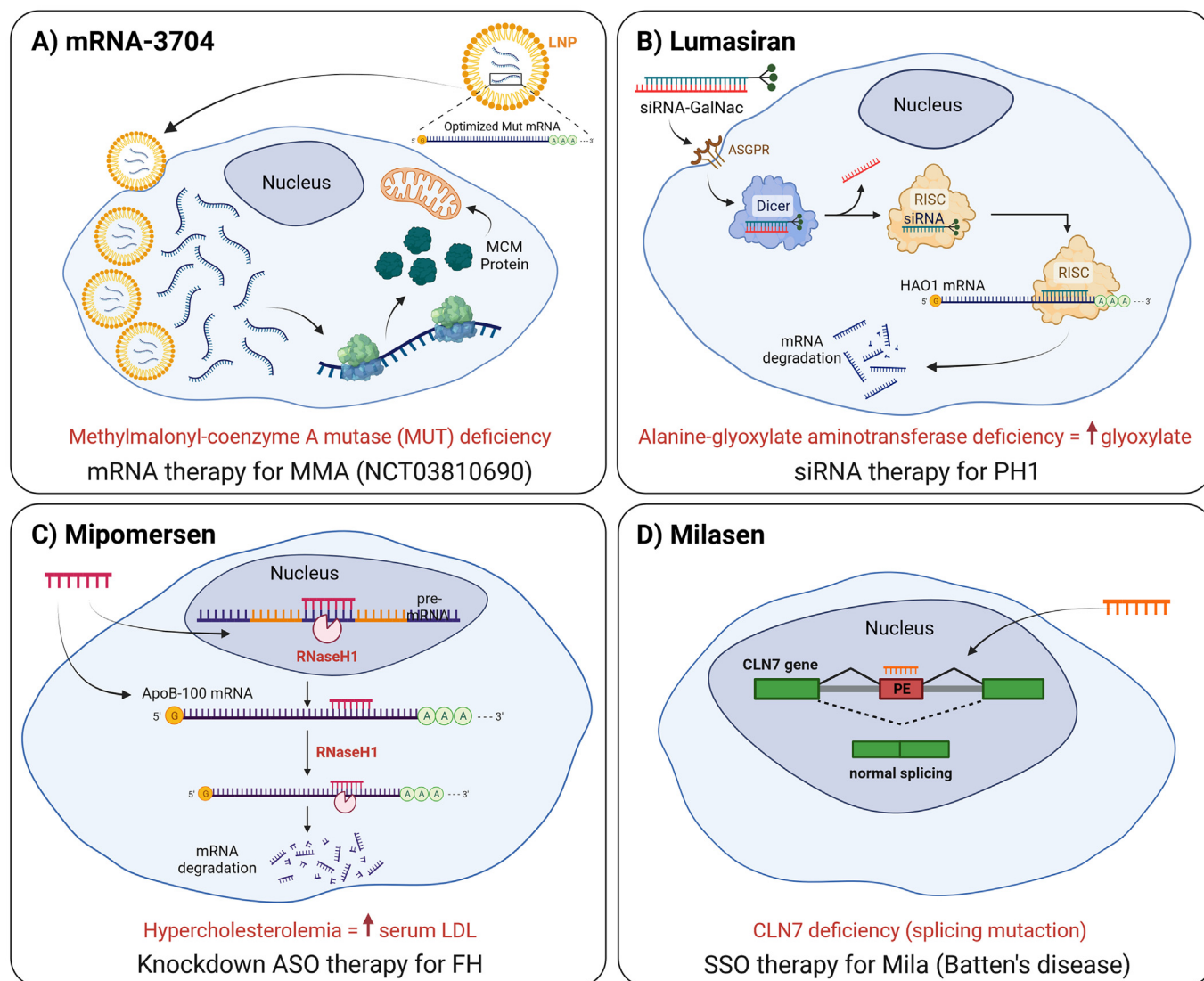
1.2. siRNA therapy

RNA interference (RNAi) was first discovered by Fire and Mello in 1998 [22] and quickly developed as a biological tool for the post-transcriptional silencing of specific genes. Its mechanism is triggered by the action of small interfering RNAs (siRNAs), double-stranded RNAs typically 20–27 base pairs in length with 3 nucleotide overhangs, after being processed by the Dicer enzyme from precursor double stranded RNAs. The antisense strand (also called the guide strand) binds with perfect complementary to its target mRNA sequence leading to its degradation through the RNA induced silencing complex (RISC) in the cytoplasm [23]. Over recent years, many studies have provided the proof of concept of the therapeutic potential of exogenous synthetic siRNAs, chemically modified to enhance stability and prevent nuclease mediated degradation. To date, four siRNA drugs have been approved (Table 1) and clinical trials are ongoing for several disorders [6].

Different approaches have proven successful for liver delivery of therapeutic siRNAs: LNP and conjugation to triantennary *N*-acetylgalactosamine (GalNAc) ligand that binds to the asialoglycoprotein receptor (ASGPR) in hepatocytes, both for systemic administration. The first LNP-RNAi drug was approved in 2018 to treat polyneuropathy in patients with hereditary amyloidosis transthyretin related (hATTR amyloidosis, OMIM #105210) and three GalNac-conjugated RNAi drugs have been approved to date, for treating AHP, PH1 and hypercholesterolemia (familial and non-familial) (Table 1) [9]. This last case represents the first example of an approved RNA drug developed to treat a prevalent disease, which may become the first widely used siRNA drug, after reassuring safety and efficacy data from clinical trials on thousands of patients [9].

In the metabolic disease PH1 there is an increase in hepatic oxalate production caused by a deficiency of the liver peroxisomal enzyme alanine-glyoxylate aminotransferase, that converts glyoxylate to glycine. Glyoxylate is produced from glycolate by the glycolate oxidase (GO) enzyme. Excess glyoxylate in PH1 is converted to oxalate that is delivered to the kidneys for excretion, causing hyperoxaluria and

Fig. 2. Mechanism of action for selected RNA-based therapeutics approved or in clinical trials to treat IEM. A) mRNA-3704 is an mRNA therapy for methylmalonic aciduria (MMA). Optimized methylmalonyl-coenzyme A mutase (MUT) mRNA formulated in lipid nanoparticles (LNPs) is delivered into hepatocytes restoring mitochondrial methylmalonyl-CoA mutase (MCM). B) Lumasiran is a double-stranded siRNA conjugated with GalNAc ligand for effective uptake by hepatocytes *via* the asialoglycoprotein receptor (ASGPR) for treatment of primary hyperoxaluria type 1 (PH1), characterized by high glyoxylate which cannot be metabolized due to the deficiency of alanine-glyoxylate aminotransferase. After uptake, siRNA is processed by Dicer and incorporated into the RNA induced silencing complex (RISC) that breaks down its target, hydroxyacid oxidase 1 (HAO1) mRNA, coding for the GO enzyme that produces glyoxylate. C) Mipomersen is a gapmer antisense oligonucleotide (ASO) recruiting RNase-H1 to its target, apolipoprotein B-100 (apoB-100) mRNA, to degrade it thus reducing lipoprotein production, indicated for familial hypercholesterolemia (FH). D) Milasen is a splice-switching antisense oligonucleotide (SSO) designed to bind a splice enhancer in intron 6 of the CLN7 gene inhibiting pseudoexon (PE) inclusion and restoring normal splicing, designed and tested for a single patient, Mila, with neuronal ceroid lipofuscinosis (NCL), a form of Batten disease. Figure generated using BioRender.com.

progressive kidney disease, while continued calcium oxalate deposition in tissues results in severe end-organ damage. Lumasiran is a GalNac-siRNA approved in 2020 that targets the HAO1 mRNA coding for the GO enzyme, thus decreasing the production of glyoxylate and oxalate in PH1 patients and lowering plasma and urinary oxalate levels (Fig. 2B) [24].

In AHP there is an accumulation of the neurotoxic porphyrin precursors delta-aminolevulinic acid and porphobilinogen, caused by enhanced activity of aminolevulinate synthase 1 (ALAS1), in the hepatic pathway of heme biosynthesis. The disease is characterized by acute, potentially life-threatening neurovisceral attacks. Givosiran, approved in 2019, is a GalNac-siRNA that targets ALAS1 mRNA, thus silencing the expression of the gene and decreasing the accumulation of neurotoxic porphyrin precursors in patients, reducing the number of acute attacks and improving symptoms of the disease, although personalized dosing regimens are required [25].

1.3. saRNA therapy

Small activating RNA (saRNA) is a type of double stranded (ds) RNA that induces target gene upregulation, thus constituting a promising option for treating human diseases. In 2006, Li et al. showed that transfection of a dsRNAs targeting the promoter of human E-cadherin, p21 and VEGF genes activated their transcription [26]. The mechanism was shown to be sequence specific and dependent on Argonaute 2 (Ago2) [27]. Epigenetic changes and spatial conformation of specific sequences in the mRNA lead to transcription activation [28]. In terms of therapeutic development, saRNAs targeting multiple cancers have been developed in preclinical models and there is an ongoing clinical trial with an saRNA targeting C/EBP for liver cancer [29]. saRNAs have the potential of treating genetic diseases characterized by haploinsufficiency or hypomorphic alleles, thus many IMD can be envisaged to benefit from this strategy in the future.

1.4. Antisense oligonucleotides (ASO)

Apart from siRNAs, the other post-translational mechanism of gene silencing broadly used for therapeutic purposes involves the use of RNAse-H1 recruiting ASOs. RNASe H1 cleaves RNA in RNA/DNA duplexes and is present both in the cytosol and in the nucleus, in contrast to the RISC complex elicited by siRNAs which is localized in the cytoplasm [30]. In first-generation ASO, phosphorothioate (PS) linkages within the ASO backbone were introduced in order to avoid nuclease degradation. Since then, extensive progress was made in ASO chemistry to enhance stability and potency, resulting in second generation ASO that include common ribose 2′-O-methyl (2′-OMe) and 2′-O-methoxyethyl (2′-MOE) modifications and further third-generation ASO that alter nucleoside conformation, such as in locked nucleic acids (LNA), constrained ethyl nucleoside analogues (cEt), tricyclo-DNA (tcDNA), or use of alternative chemistries such as phosphorodiamidate-modified morpholino oligomers (PMOs) [4,30]. Fully modified ASOs do not recruit RNase H1, therefore, for this purpose, gapmer ASOs are designed that have a central gap of DNA nucleotides with a PS backbone flanked with modified nucleotides on both sides [30]. Gapmers can be used to downregulate genes in allele independent or allele-specific manner.

To date, four RNASe H1 recruiting ASO have received approval from one or more regulatory agencies, the first was a PS DNA-based ASO developed for treating CMV retinitis patients (Fomivirsen) (Table 1). Subsequently approved were allele independent drugs for treatment of familial hypercholesterolemia (Mipomersen targeting ApoB lipoprotein mRNA) (Fig. 2C) and of familial chylomicronemia syndrome, hypertriglyceridemia and familial partial lipodystrophy (Volanesorsen, targeting apolipoprotein CIII mRNA). Both are PS 2'-MOE gapmers, that effectively lower the levels of specific lipids increased in these diseases [31]. In contrast, Inotersen is a gapmer designed to specifically target the mutant TTR mRNA encoding a transthyretin protein with a dominant-negative effect in autosomal dominant hATTR (Table 1) [32].

The other type of therapeutic ASO in the market are fully modified and use a non RNASe H1-dependent mechanism for splicing modulation, whereby the ASO (commonly named as splice-switching antisense oligonucleotide or SSO) targets, and thus blocks, binding sites for spliceosomal components or for splice regulatory factors. The mechanism implies steric hindrance for RNA binding proteins to access their binding motifs and results in an alteration in pre-mRNA splicing in the nucleus. This is used for a variety of purposes with a therapeutic aim: promoting exon inclusion or exon skipping, preventing aberrant cryptic splice site usage, modifying alternative splicing and preventing pseudoexon inclusion [33].

Dominski and Kole were the first to demonstrate that ASO could be used to target splicing in 1993, correcting an aberrant splicing defect in the beta-globin gene causing beta-thalassemia [34]. Correct splicing relies on the presence of conserved 3′ and 5′ splice sites at the intron-exon junctions as well as of other cis-acting elements such as exonic/intronic splicing enhancers (ESE, ISE) or exonic/intronic splicing silencers (ESS, ISS), that participate in a finely balanced interplay in exon recognition. Depending on the region targeted by the SSO, the splicing outcome will be different. The SSO Nusinersen (commercial name Spinraza) acts by blocking an ISS in intron 7 of the SMN2 gene, thus favouring exon 7 inclusion and the production of a functional SMN protein as therapy for spinal muscular atrophy (SMA). This is by far the most successful SSO to date, both clinically (treated patients show significant improvements in motor skills and muscle function) and commercially, achieving more than $4.5 billion in sales by the end of 2019 [1]. In Duchenne muscular dystrophy (DMD), several approved SSOs target ESEs in specific exons, promoting exon skipping to produce a mature transcript with a restored open reading frame coding for a partially functional dystrophin (Table 1) [4].

SSOs have also been used to block cryptic splice sites or activated pseudoexons preventing their aberrant inclusion in the mRNA. Many pseudoexons are transposable sequence elements and are frequently activated by point mutations causing human disease [35]. Blocking with SSO the splice sites or enhancer elements in the pseudoexon restores normal splicing and this has been proven successful in many preclinical studies in different diseases, including IEM [33,36,37]. Recently, an SSO (Milasen) (Fig. 2D) targeting an activated pseudoexon in the *CLN7* gene causing neuronal ceroid lipofuscinosis (NCL, OMIM #610951, a type of Batten's disease) was developed and approved by the FDA for clinical testing just within one year, and, most extraordinary, it was designed for just one patient (Mila) carrying this private mutant allele (Table 1) [38]. This marked the beginning of $n = 1$ trials for patients with ultra-rare mutations, extreme examples of personalized medicine. Similarly, the RNA drug Jacifusen was designed and tested in a patient (Jaci Hermstad) suffering from amyotrophic lateral sclerosis with mutations in the *FUS* gene (Table 1). Unfortunately, both Mila and Jaci died recently. However, Jacifusen is currently being tested in a phase III trial for patients with the same disease (NCT04768972), exemplifying that these ASO based individualized treatments may, sometimes, be extended to other patients. Different academic groups and private foundations in several countries, in collaboration with industrial partners, are since then working to develop such customized ASO using non-profit model approaches [39]. Very recently, a worldwide initiative for development of $n = 1$ RNA therapies has been started (https://www.n1collaborative.org/).

Another therapeutic approach of SSO in development is the so called "targeted augmentation of nuclear gene output" (TANGO) [40]. Many disease-associated genes undergo non-productive splicing, due for example to residual aberrant incorporation of pseudoexons during the splicing process. SSO targeting these pseudoexons reduce their inclusion, resulting in an increase in functional mRNA and protein levels in monogenic diseases characterized by haploinsufficiency, as in Dravet syndrome [41] or in patients carrying hypomorphic alleles which is quite frequent in many IEM. In fact, PA resulting from *PCCA* gene defects was described as a candidate gene for application of this novel approach [40], due to the presence of a naturally occurring alternative splicing event including a pseudoexon that disrupts the open reading frame and induces nonsense-mediated mRNA decay [42]. In theory, the approach could be applicable to other IEM with naturally occurring pseudoexon inclusion events and missense mutations with residual activity [37,43].

In addition, research is ongoing with fully modified ASO acting as steric blockers for different therapeutic aims, including targeting translational initiation sites to inhibit protein production, or inhibiting sequestration of RNA binding proteins by a mutant gain-of-function toxic RNA, as in myotonic dystrophy [44].

1.5. Aptamers

Aptamers are short single-stranded nucleic acids that act by binding proteins due to their tertiary structure, thus mimicking antibodies, although with considerable advantages in terms of size, structural flexibility, superior target recognition and easier synthesis [45,46]. They are typically identified after iterative enrichment rounds from a random pool of degenerate oligonucleotide sequences and selection on the basis of their binding or functional activities (an *in vitro* procedure to select functional nucleic acids, termed SELEX) [47]. Only one aptamer-based drug has received approval so far, pegaptanib (Macugen), for the prevention of age-related macular degeneration (Table 1), although many others are now being developed [45].

1.6. miRNA-based therapies

miRNAs are short noncoding RNAs, 20–24 nucleotides in length, that act regulating gene expression by base pairing with target mRNAs in the cytoplasm, resulting in their degradation or in inhibition of translation [48]. miRNAs are generated by RNA polymerase II as hairpin stem-loop precursors (pri-miRNAs), which are first processed in the nucleus

by Drosha/DGCR8 or by alternative mechanisms, resulting in a ~ 70 nucleotides pre-miRNA. Following nuclear processing, pre-miRNAs are transported to the cytoplasm and further processed by Dicer into a ds-miRNA which is loaded into AGO proteins. One of the strands is degraded and the other remains as the mature miRNA. Gene silencing is elicited through the RISC complex mediated by base pairing of the miRNA with the 3′ untranslated regions of target mRNAs [48]. The first miRNA, lin-4, was discovered in 1993 in *Caenorhabditis elegans* [49] and, since then, thousands of miRNAs have been identified both in humans and other species. There is a tissue-specific expression of miRNAs and their functional importance in normal biological processes is to date well established, as is the link between their dysregulation and pathological processes operating in different diseases, including cancer, neurodegenerative, cardiovascular diseases, diabetes and IEM [50,51]. miRNA-based therapeutics can be categorized into two types: miRNA inhibitors (antimiRs or antagomiRs), which are ASO targeting the corresponding miRNA, and miRNA mimics, precursor double-stranded RNA molecules that when added exogenously, follow the same mechanism of gene silencing as miRNAs. To date, there are no miRNA-based drugs on the market, although several promising candidates are being developed or are in clinical trials, the vast majority for cancer, but also for monogenic diseases, *e.g.* an antimiR-23b for myotonic dystrophy that acts by increasing MBNL protein levels thus reversing the disease phenotype [52], or an antimiR-21 to treat Alport Syndrome (NCT02855268).

1.7. lncRNAs

lncRNAs are noncoding RNAs longer than 200 nucleotides in length, that share some characteristics with mRNAs (they are often 5′ capped, 3′ polyadenylated and undergo splicing) but have very low protein coding potential. As with miRNAs, they also exhibit tissue specificity and emerging evidence points to their relevant functional roles in normal physiology. Altered expression of lncRNAs have been associated with cancer, metabolic, developmental and cardiovascular diseases and their mechanism of action regulating gene expression includes recruitment of chromatin-modifying complexes, antisense transcription, splicing regulation, miRNA interaction or protein scaffolding, among others [53,54].

Recently, the lncRNA *HULC* (*Pair* in mice) was discovered to be involved in the regulation of phenylalanine hydroxylase (PAH) activity, by virtue of a direct interaction with the regulatory region of the enzyme, facilitating the interaction with substrate and cofactor [15]. Depletion of this lncRNA in mice led to a characteristic PKU phenotype and administration of a *HULC* lncRNA mimic was found to enhance residual enzyme activity of the common p.R408W PAH variant, thus providing the first example of a lncRNA linked to the pathophysiology of an IEM [15]. These observations suggest potential therapeutic application of this lncRNA mimics for the future treatment of some PKU patients [55] and pave the way for the study of the role of lncRNAs in the pathophysiology of other IEM and of novel lncRNA-based applications for their treatment.

2. Conclusions and future perspectives

Although currently there are only around a dozen therapeutic RNA drugs (plus two mRNA vaccines) formally approved for clinical use, there are many new such drugs in the pipeline for a plethora of both rare and common diseases. With the exception of aptamers and some lncRNAs, as in the case of *HULC*, RNA drugs interact with different nucleic acids, thanks to sequence specific Watson–Crick base pairing. This makes them easy to design to bind specific targets, less likely to cause side effects and are in many ways considered as one of the leading candidates for the next wave of precision medicine. There are still significant challenges in developing efficient RNA drugs for therapeutic purposes, mainly related to targeted delivery and endosomal escape after

cellular uptake, which have been reviewed elsewhere [4,10]. However, they have already proven to be an ideal modality to meet clinical unmet needs. They have even offered hope for ultrarare disease patients due to their easy and fast individualized development, with $n = 1$ trials that have re-evaluated the speed and type of safety studies and regulatory requirements [56].

There appears to be no limit to RNA therapies [8]. Recent data with engineered tRNAs that can suppress premature termination codons suggest the exciting possibility that a single tRNA drug may be used to treat multiple diseases, indicated for patients with the same type of nonsense mutations, that overall account for an estimated 11% of all pathogenic variants [57,58]. Indeed, all the research efforts and the investment into mRNA solutions are well worth it, and IEM will surely benefit from current and future development of all RNA therapeutic strategies.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] F. Wang, T. Zuroske, J.K. Watts, RNA therapeutics on the rise, Nat. Rev. Drug Discov. 19 (2020) 441–442.

[2] S.T. Crooke, X.H. Liang, R.M. Crooke, B.F. Baker, R.S. Geary, Antisense drug discovery and development technology considered in a pharmacological context, Biochem. Pharmacol. 189 (2021) 114196.

[3] S.T. Crooke, B.F. Baker, R.M. Crooke, X.H. Liang, Antisense technology: an overview and prospectus, Nat. Rev. Drug Discov. 20 (2021) 427–453.

[4] S.M. Hammond, A. Aartsma-Rus, S. Alves, S.E. Borgos, R.A.M. Buijsen, R.W.J. Collin, G. Covello, M.A. Denti, L.R. Desviat, L. Echevarria, C. Foged, G. Gaina, A. Garanto, A.T. Goyenvalle, M. Guzowska, I. Holodnuka, D.R. Jones, S. Krause, T. Lehto, M. Montolio, W. Van Roon-Mom, V. Arechavala-Gomeza, Delivery of oligonucleotide-based therapeutics: challenges and opportunities, EMBO Mol. Med. 13 (2021) e13243.

[5] A. Gupta, J.L. Andresen, R.S. Manan, R. Langer, Nucleic acid delivery for therapeutic applications, Adv. Drug Deliv. Rev. 178 (2021) 113834.

[6] Y. Zhao, R. Shu, J. Liu, The development and improvement of ribonucleic acid therapy strategies, Mol. Ther. Nucleic Acids 26 (2021) 997–1013.

[7] T. Gokirmak, M. Nikan, S. Wiechmann, T.P. Prakash, M. Tanowitz, P.P. Seth, Overcoming the challenges of tissue delivery for oligonucleotide therapeutics, Trends Pharmacol. Sci. 42 (2021) 588–604.

[8] T.R. Damase, R. Sukhovershin, C. Boada, F. Taraballi, R.I. Pettigrew, J.P. Cooke, The limitless future of RNA therapeutics, Front. Bioeng. Biotechnol. 9 (2021) 628137.

[9] D.R. Corey, M.J. Damha, M. Manoharan, Challenges and opportunities for nucleic acid therapeutics, Nucleic Acid Ther. 32 (2022) 8–13.

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

[11] F. Halloy, A. Biscans, K.E. Bujold, A. Debacker, A.C. Hill, A. Lacroix, O. Luige, R. Stromberg, L. Sundstrom, J. Vogel, A. Ghidini, Innovative developments and emerging technologies in RNA therapeutics, RNA Biol. 19 (2022) 313–332.

[12] J.M. Sasso, B.J.B. Ambrose, R. Tenchov, R.S. Datta, M.T. Basel, R.K. DeLong, Q.A. Zhou, The Progress and promise of RNA medicine horizontal line an arsenal of targeted treatments, J. Med. Chem. 65 (2022) 6975–7015.

[13] H. Zogg, R. Singh, S. Ro, Current advances in RNA therapeutics for human diseases, Int. J. Mol. Sci. 23 (2022).

[14] C.R. Ferreira, S. Rahman, M. Keller, J. Zschocke, I.A. Group, An international classification of inherited metabolic disorders (ICIMD), J. Inherit. Metab. Dis. 44 (2021) 164–177.

[15] Y. Li, Z. Tan, Y. Zhang, Z. Zhang, Q. Hu, K. Liang, Y. Jun, Y. Ye, Y.C. Li, C. Li, L. Liao, J. Xu, Z. Xing, Y. Pan, S.S. Chatterjee, T.K. Nguyen, H. Hsiao, S.D. Egranov, N. Putluri, C. Coarfa, D.H. Hawke, P.H. Gunaratne, K.L. Tsai, L. Han, M.C. Hung, G.A. Calin, F. Namour, J.L. Gueant, A.C. Muntau, N. Blau, V.R. Sutton, M. Schiff, F. Feillet, S. Zhang, C. Lin, L. Yang, A noncoding RNA modulator potentiates phenylalanine metabolism in mice, Science 373 (2021) 662–673.

[16] J.A. Wolff, R.W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani, P.L. Felgner, Direct gene transfer into mouse muscle in vivo, Science 247 (1990) 1465–1468.

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

[18] U. Sahin, K. Kariko, O. Tureci, mRNA-based therapeutics–developing a new class of drugs, Nat. Rev. Drug Discov. 13 (2014) 759–780.

[19] P. Berraondo, P.G.V. Martini, M.A. Avila, A. Fontanellas, Messenger RNA therapy for rare genetic metabolic diseases, Gut 68 (2019) 1323–1330.

[20] L. Jiang, J.S. Park, L. Yin, R. Laureano, E. Jacquinet, J. Yang, S. Liang, A. Frassetto, J. Zhuo, X. Yan, X. Zhu, S. Fortucci, K. Hoar, C. Mihai, C. Tunkey, V. Presnyak, K.E. Benenato, C.M. Lukacs, P.G.V. Martini, L.T. Guey, Dual mRNA therapy restores metabolic function in long-term studies in mice with propionic acidemia, Nat. Commun. 11 (2020) 5339.

[21] X. Zhu, L. Yin, M. Theisen, J. Zhuo, S. Siddiqui, B. Levy, V. Presnyak, A. Frassetto, J. Milton, T. Salerno, K.E. Benenato, J. Milano, A. Lynn, S. Sabnis, K. Burke, G. Besin, C.M. Lukacs, L.T. Guey, P.F. Finn, P.G.V. Martini, Systemic mRNA therapy for the treatment of Fabry disease: preclinical studies in wild-type mice, Fabry mouse model, and wild-type non-human Primates, Am. J. Hum. Genet. 104 (2019) 625–637.

[22] A. Fire, S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, C.C. Mello, Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*, Nature 391 (1998) 806–811.

[23] T.A. Rand, K. Ginalski, N.V. Grishin, X. Wang, Biochemical identification of Argonaute 2 as the sole protein required for RNA-induced silencing complex activity, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 14385–14389.

[24] D.J. Sas, D. Magen, W. Hayes, H. Shasha-Lavsky, M. Michael, I. Schulte, A.L. Sellier-Leclerc, J. Lu, A. Seddighzadeh, B. Habtemariam, T.L. McGregor, K.P. Fujita, Y. Frishberg, I.-B. Workgroup, Phase 3 trial of lumasiran for primary hyperoxaluria type 1: a new RNAi therapeutic in infants and young children, Genet. Med. 24 (2022) 654–662.

[25] A. Poli, C. Schmitt, B. Moulouel, A. Mirmiran, N. Talbi, S. Riviere, D. Cerutti, I. Bouchoule, A. Faivre, V. Grobost, C. Douillard, F. Duchene, V. Fiorentino, T. Dupre, H. Manceau, K. Peoc'h, H. Puy, T. Lefebvre, L. Gouya, Givosiran in acute intermittent porphyria: a personalized medicine approach, Mol. Genet. Metab. 135 (2022) 206–214.

[26] L.C. Li, S.T. Okino, H. Zhao, D. Pookot, R.F. Place, S. Urakami, H. Enokida, R. Dahiya, Small dsRNAs induce transcriptional activation in human cells, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 17337–17342.

[27] V. Portnoy, V. Huang, R.F. Place, L.C. Li, Small RNA and transcriptional upregulation Wiley Interdiscip rev, RNA 2 (2011) 748–760.

[28] B.A. Janowski, S.T. Younger, D.B. Hardy, R. Ram, K.E. Huffman, D.R. Corey, Activating gene expression in mammalian cells with promoter-targeted duplex RNAs, Nat. Chem. Biol. 3 (2007) 166–173.

[29] S. Yoon, J.J. Rossi, Therapeutic potential of small activating RNAs (saRNAs) in human cancers, Curr. Pharm. Biotechnol. 19 (2018) 604–610.

[30] S.T. Crooke, X.H. Liang, B.F. Baker, R.M. Crooke, Antisense technology: a review, J. Biol. Chem. 296 (2021) 100416.

[31] T. Aslesh, T. Yokota, Development of antisense oligonucleotide Gapmers for the treatment of dyslipidemia and lipodystrophy, Methods Mol. Biol. 2176 (2020) 69–85.

[32] A. Aimo, V. Castiglione, C. Rapezzi, M. Franzini, G. Panichella, G. Vergaro, J. Gillmore, M. Fontana, C. Passino, M. Emdin, RNA-targeting and gene editing therapies for transthyretin amyloidosis, Nat. Rev. Cardiol. (2022) https://doi.org/10.1038/s41569-022-00683-z In press.

[33] E.C. Kuijper, A.J. Bergsma, W. Pijnappel, A. Aartsma-Rus, Opportunities and challenges for antisense oligonucleotide therapies, J. Inherit. Metab. Dis. 44 (1) (2020) 72–87, https://doi.org/10.1002/jimd.12251.

[34] Z. Dominski, R. Kole, Restoration of correct splicing in thalassemic pre-mRNA by antisense oligonucleotides, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 8673–8677.

[35] U.S.S. Petersen, T.K. Doktor, B.S. Andresen, Pseudoexon activation in disease by non-splice site deep intronic sequence variation - wild type pseudoexons constitute high-risk sites in the human genome, Hum. Mutat. 43 (2022) 103–127.

[36] R. Sangermano, A. Garanto, M. Khan, E.H. Runhart, M. Bauwens, N.M. Bax, L.I. van den Born, M.I. Khan, S.S. Cornelis, J. Verheij, J.R. Pott, A. Thiadens, C.C.W. Klaver, B. Puech, I. Meunier, S. Naessens, G. Arno, A. Fakin, K.J. Carss, F.L. Raymond, A.R. Webster, C.M. Dhaenens, H. Stohr, F. Grassmann, B.H.F. Weber, C.B. Hoyng, E. De Baere, S. Albert, R.W.J. Collin, F.P.M. Cremers, Deep-intronic ABCA4 variants explain missing heritability in Stargardt disease and allow correction of splice defects by antisense oligonucleotides, Genet. Med. 21 (2019) 1751–1760.

[37] B. Perez, L. Rodriguez-Pascau, L. Vilageliu, D. Grinberg, M. Ugarte, L.R. Desviat, Present and future of antisense therapy for splicing modulation in inherited metabolic disease, J. Inherit. Metab. Dis. 33 (2010) 397–403.

[38] J. Kim, C. Hu, C. Moufawad El Achkar, L.E. Black, J. Douville, A. Larson, M.K. Pendergast, S.F. Goldkind, E.A. Lee, A. Kuniholm, A. Soucy, J. Vaze, N.R. Belur, K. Fredriksen, I. Stojkovska, A. Tsytsykova, M. Armant, R.L. DiDonato, J. Choi, L. Cornelissen, L.M. Pereira, E.F. Augustine, C.A. Genetti, K. Dies, B. Barton, L. Williams, B.D. Goodlett, B.L. Riley, A. Pasternak, E.R. Berry, K.A. Pflock, S. Chu, C. Reed, K. Tyndall, P.B. Agrawal, A.H. Beggs, P.E. Grant, D.K. Urion, R.O. Snyder, S.E. Waisbren, A. Poduri, P.J. Park, A. Patterson, A. Biffi, J.R. Mazzulli, O. Bodamer, C.B. Berde, T.W. Yu, Patient-customized oligonucleotide therapy for a rare genetic disease, N. Engl. J. Med. 381 (2019) 1644–1652.

[39] S.T. Crooke, Meeting the needs of patients with ultrarare diseases, Trends Mol. Med. 28 (2022) 87–96.

[40] K.H. Lim, Z. Han, H.Y. Jeon, J. Kach, E. Jing, S. Weyn-Vanhentenryck, M. Downs, A. Corrionero, R. Oh, J. Scharner, A. Venkatesh, S. Ji, G. Liau, B. Ticho, H. Nash, I.

[41] Z. Han, C. Chen, A. Christiansen, S. Ji, Q. Lin, C. Anumonwo, C. Liu, S.C. Leiser, I. Meena, G. Aznarez, L.L. Isom Liau, Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome, Sci. Transl. Med. 12 (2020).

[42] E. Campeau, L. Dupuis, D. Leclerc, R.A. Gravel, Detection of a normally rare transcript in propionic acidemia patients with mRNA destabilizing mutations in the PCCA gene, Hum. Mol. Genet. 8 (1999) 107–113.

[43] A. Martinez-Pizarro, F. Leal, L.L. Holm, T.K. Doktor, U.S.S. Petersen, M. Bueno, B. Thony, B. Perez, B.S. Andresen, L.R. Desviat, Antisense oligonucleotide rescue of deep-intronic variants activating pseudoexons in the PTS gene, Nucleic Acid Ther. (2022) In press.

[44] S.J. Overby, E. Cerro-Herreros, B. Llamusi, R. Artero, RNA-mediated therapies in myotonic dystrophy, Drug Discov. Today 23 (2018) 2013–2022.

[45] A.D. Keefe, S. Pai, A. Ellington, Aptamers as therapeutics, Nat. Rev. Drug Discov. 9 (2010) 537–550.

[46] S. Yoon, J.J. Rossi, Aptamers: uptake mechanisms and intracellular applications, Adv. Drug Deliv. Rev. 134 (2018) 22–35.

[47] K. Sefah, D. Shangguan, X. Xiong, M.B. O'Donoghue, W. Tan, Development of DNA aptamers using cell-SELEX, Nat. Protoc. 5 (2010) 1169–1185.

[48] S. Jonas, E. Izaurralde, Towards a molecular understanding of microRNA-mediated gene silencing, Nat. Rev. Genet. 16 (2015) 421–433.

[49] R.C. Lee, R.L. Feinbaum, V. Ambros, The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14, Cell 75 (1993) 843–854.

[50] A. Rivera-Barahona, B. Perez, E. Richard, L.R. Desviat, Role of miRNAs in human disease and inborn errors of metabolism, J. Inherit. Metab. Dis. 40 (2017) 471–480.

[51] A. Fulgencio-Covian, E. Alonso-Barroso, A.J. Guenzel, A. Rivera-Barahona, M. Ugarte, B. Perez, M.A. Barry, C. Perez-Cerda, E. Richard, L.R. Desviat, Pathogenic implications of dysregulated miRNAs in propionic acidemia related cardiomyopathy, Transl. Res. 218 (2020) 43–56.

[52] S.J. Overby, E. Cerro-Herreros, I. Gonzalez-Martinez, M.A. Varela, D. Seoane-Miraz, Y. Jad, R. Raz, T. Moller, M. Perez-Alonso, M.J. Wood, B. Llamusi, R. Artero, Proof of concept of peptide-linked blockmiR-induced MBNL functional rescue in myotonic dystrophy type 1 mouse model, Mol. Ther. Nucleic Acids 27 (2022) 1146–1155.

[53] J.K. DiStefano, The emerging role of long noncoding RNAs in human disease, Methods Mol. Biol. 1706 (2018) 91–110.

[54] P. Sparber, A. Filatova, M. Khantemirova, M. Skoblov, The role of long non-coding RNAs in the pathogenesis of hereditary diseases, BMC Med. Genet. 12 (2019) 42.

[55] C. Lin, Y. Li, E. Zhang, F. Feillet, S. Zhang, N. Blau, Importance of the long non-coding RNA (lncRNA) transcript HULC for the regulation of phenylalanine hydroxylase and treatment of phenylketonuria, Mol. Genet. Metab. 135 (2022) 171–178.

[56] J. Kaiser, RNA drugs custommade for rare diseases face wider test, Science 374 (2021) 672.

[57] J.D. Lueck, J.S. Yoon, A. Perales-Puchalt, A.L. Mackey, D.T. Infield, M.A. Behlke, M.R. Pope, D.B. Weiner, W.R. Skach, P.B. McCray Jr., C.A. Ahern, Engineered transfer RNAs for suppression of premature termination codons, Nat. Commun. 10 (2019) 822.

[58] E. Dolgin, tRNA therapeutics burst onto startup scene, Nat. Biotechnol. 40 (2022) 283–286.

[59] C. Marwick, First "antisense" drug will treat CMV retinitis, JAMA 280 (1998) 871.

[60] P. Hair, F. Cameron, K. McKeage, Mipomersen sodium: first global approval, Drugs 73 (2013) 487–493.

[61] M.D. Benson, M. Waddington-Cruz, J.L. Berk, M. Polydefkis, P.J. Dyck, A.K. Wang, V. Plante-Bordeneuve, F.A. Barroso, G. Merlini, L. Obici, M. Scheinberg, T.H. Brannagan 3rd, W.J. Litchy, C. Whelan, B.M. Drachman, D. Adams, S.B. Heitner, I. Conceicao, H.H. Schmidt, G. Vita, J.M. Campistol, J. Gamez, P.D. Gorevic, E. Gane, A.M. Shah, S.D. Solomon, B.P. Monia, S.G. Hughes, T.J. Kwoh, B.W. McEvoy, S.W. Jung, B.F. Baker, E.J. Ackermann, M.A. Gertz, T. Coelho, Inotersen treatment for patients with hereditary transthyretin amyloidosis, N. Engl. J. Med. 379 (2018) 22–31.

[62] G. Kolovou, V. Kolovou, N. Katsiki, Volanesorsen: a new era in the treatment of severe hypertriglyceridemia, J. Clin. Med. (2022) 11.

[63] E.W. Ng, D.T. Shima, P. Calias, E.T. Cunningham Jr., D.R. Guyer, A.P. Adamis, Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease, Nat. Rev. Drug Discov. 5 (2006) 123–132.

[64] D. Adams, A. Gonzalez-Duarte, W.D. O'Riordan, C.C. Yang, M. Ueda, A.V. Kristen, I. Tournev, H.H. Schmidt, T. Coelho, J.L. Berk, K.P. Lin, G. Vita, S. Attarian, V. Plante-Bordeneuve, M.M. Mezei, J.M. Campistol, J. Buades, T.H. Brannagan 3rd, B.J. Kim, J. Oh, Y. Parman, Y. Sekijima, P.N. Hawkins, S.D. Solomon, M. Polydefkis, P.J. Dyck, P.J. Gandhi, S. Goyal, J. Chen, A.L. Strahs, S.V. Nochur, M.T. Sweetser, P.P. Garg, A.K. Vaishnaw, J.A. Gollob, O.B. Suhr, Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis, N. Engl. J. Med. 379 (2018) 11–21.

[65] F. Hughes-Hubley, M. Iskander, A. Cheng-Lai, W.H. Frishman, J. Nawarskas, Inclisiran: small interfering ribonucleic acid injectable for the treatment of hyperlipidemia, Cardiol. Rev. 30 (2022) 214–219.

[66] J.S. Charleston, F.J. Schnell, J. Dworzak, C. Donoghue, S. Lewis, L. Chen, G.D. Young, A.J. Milici, J. Voss, U. DeAlwis, B. Wentworth, L.R. Rodino-Klapac, Z. Sahenk, D. Frank, J.R. Mendell, Eteplirsen treatment for Duchenne muscular dystrophy: exon skipping and dystrophin production, Neurology 90 (2018) e2146–e2154.

[67] Y.A. Heo, Golodirsen: first approval, Drugs 80 (2020) 329–333.

[68] S. Dhillon, Viltolarsen: first approval, Drugs 80 (2020) 1027–1031.

[69] M. Shirley, Casimersen: first approval, Drugs 81 (2021) 875–879.

[70] D.R. Corey, Nusinersen, an antisense oligonucleotide drug for spinal muscular atrophy, Nat. Neurosci. 20 (2017) 497–499.