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RNA-based drugs: current, imminent and possible therapeutic applications

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ABSTRACT

In a relatively brief period, the mRNA COVID-19 vaccines have saved millions of lives and have considerably contributed to return to normality after the pandemic. More broadly, the development of RNA-based drugs represents a real paradigm shift with promising therapeutic applications. Besides their safety and efficacy, RNA-based drugs are essentially easy to design and manufactured and may therefore be cost effective. At the pharmacological level, the development of RNA-based drugs marks a breakthrough because these drugs can reach previously "undruggable" pharmacological targets. This clearly represents a step toward the possible establishment of personalized treatments for several difficult-to-treat diseases. This review provides an updated, critical, and comprehensive pharmacological analysis of the current RNA therapeutics landscape, including both approved RNA-based drugs and key investigational candidates. We summarize the state of clinical progress,

Abbreviations: ABC-DLBCL, activated B-cell subtype of diffuse large B-cell lymphoma; ACE-I, angiotensin converting enzyme inhibitor; AD, Alzheimer's disease; ADR, adverse drug reaction; Ago2, argonaute 2; AGT, angiotensinogen; AGXT, alanine-glyoxylate aminotransferase; AHP, hepatic porphyria; AIDS, acquired immunodeficiency syndrome; AKI, acute kidney injury; ALA, -aminolevulinic acid; ALAS1, -aminolevulinic acid synthase; ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; ANGPTL3, angiopoietin-like 3; AOC, antibody-oligonucleotide conjugate; APC, antigen-presenting cell; apo (a), apolipoprotein (a); apoC-III, apolipoprotein C-III; ARB, angiotensin receptor blocker; ASGPR, asialoglycoprotein; ASO, antisense oligonucleotides; AT1, angiotensin 1 receptor; ATC, anaplastic thyroid cancer; BBB, blood-brain barrier; CAD, coronary artery disease; circRNA, circular RNA; CMV, cytomegalovirus; CNS, central nervous system; CRISPR-Cas, clustered regularly interspaced short palindromic repeats-CRISPR-associated; CSF, cerebrospinal fluid; CTCL, cutaneous T-cell lymphoma; CVD, cardiovascular disease; CXCL12, CXC chemokine ligand 12; DC, dendritic cell; DMD, Duchenne muscular dystrophy; EBV, Epstein-Barr virus; EL, endothelial lipase; EMA, European Medicines Agency; ESA, erythropoiesis-stimulating agents; EU, European Union; FCS, familial chylomicronemia syndrome; FDA, Food and Drug Administration; FUS, fused in sarcoma; GalNAc, *N*-acetylgalactosamine; GO, glycolate oxidase; gRNA, guide RNA; HAE, hereditary angioedema; HAO1, hydroxy acid oxidase 1; hATTR, hereditary transthyretin amyloidosis; HbF, fetal hemoglobin; HbS, sickle hemoglobin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HD, Huntington's disease; HeFH, heterozygous familial hypercholesterolemia; HNRNPL, heterogeneous nuclear ribonucleoprotein L; HPV16, human papillomavirus 16; HSD17B13, hydroxysteroid 17-dehydrogenase 13; HSV, herpes simplex virus; HTT, huntingtin; IE2, immediate early region 2; IGFIIR, insulin-like growth factor 2 receptor; iNeST, individualized neoantigen specific therapy; ISS-1, intronic splice silencing site-1; LCA10, Leber congenital amaurosis type 10; LDHA, lactate dehydrogenase A; LDL, low density lipoprotein; LICA, ligand-conjugated antisense; LNA, locked nucleic acid; LNP, lipid-based nanoparticle; LPL, lipoprotein lipase; LR-MDS, lower-risk myelodysplastic syndromes; LRTD, lower respiratory tract disease; MASH, metabolic dysfunction-associated steatohepatitis; MCNS, minimal change nephrotic syndrome; MDD, major depressive disorder; MF, mycosis fungoides; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; MHRA, medicines and healthcare products regulatory agency; mHTT, mutated huntingtin; miRNA, MicroRNA; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ncRNA, non-coding RNA; NS, nephrotic syndrome; NSCLC, non-small cell lung cancer; OX40L, OX40 ligand; PCSK9, proprotein convertase subtilisin/kexin type 9; PDCD4, programmed cell death 4; PDUFA, Prescription Drug User Fee Act; PEG, polyethylene glycol; PH1, primary hyperoxaluria type 1; PKK, prekallikrein; PNP, polymer-based nanoparticles; pre-RISC, premature RNAi-induced silencing complex; PTSD, post-traumatic stress disorder; RABV, rabies virus; RNA, ribonucleic acid; RNAse H, ribonuclease H; RSV, respiratory syncytial virus; SERT, serotonin transporter; siRNA, short interfering RNAs; SMA, spinal muscular atrophy; SMN1, survival motor neuron 1; SMN2, survival motor neuron 2; SOD1, superoxide dismutase 1; SSO, splice-switching oligonucleotide; SSRI, selective serotonin reuptake inhibitors; T1D, type 1 diabetes patients; T2D, type 2 diabetes; TLR8, toll-like receptor 8; TPTE, trans-membrane phosphatase with tensin homology; tRNA, transfer RNA; TTR, transthyretin; VEGF, vascular endothelial growth factor; vLDL, very low-density lipoprotein; VOC, vaso-occlusive crisis; VWF, von Willebrand factor; wtHTT, wild-type huntingtin; ZikV, Zika virus..

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highlighting pharmacological mechanisms, challenges in drug delivery, tolerability, and clinical outcomes. Our comprehensive overview emphasizes the versatility of RNA-based drugs, illustrating their therapeutic application across various diseases such as cancer, neurodegenerative, cardiovascular, metabolic, rare genetic, and infectious diseases. Also, we uniquely explore the concept of RNA-based drugs repurposing, which may leverage shared pathophysiological mechanisms across diseases to accelerate clinical impact.

1. Background

Ribonucleic acid (RNA) therapeutics rely on the use of RNA-based drugs to modulate gene expression and protein synthesis for the prevention or treatment of various diseases. RNA therapeutics are rapidly transforming the landscape of modern medicine by offering innovative solutions for diseases that were previously difficult or impossible to treat. In contrast to small-molecules and protein-based drugs, RNA-based drugs exert their effect on the molecular mechanisms that cause disease with unprecedented precision and selectivity (Damase et al., 2021). Indeed, RNA molecules hold unique properties that enable selective interaction with proteins, transcripts, and genes that cannot be targeted by conventional small molecules or proteins. The first RNA-based therapy was developed in 1978 by Stephenson and Zamecnik, who used an antisense oligonucleotide to inhibit the replication of the Rous Sarcoma virus. Yet, it took almost 20 years to obtain approval for the Food and Drug Administration (FDA) to approve the first RNA drug (Roehr, 1998). Despite the challenging and long history of RNA molecules as drugs, they have dominated the global scenario during the COVID-19 pandemic as vaccines. The surprising success obtained during the COVID-19 pandemic has sparked a rapid advancement in RNA therapeutics, attracting significant investment from global pharmaceutical companies (Zhou et al., 2023) and leading to the emergence of innovative RNA drugs entering clinical trials at a rising rate (Curreri et al., 2023).

Due to their versatility, RNA-based drugs open new therapeutic avenues for a broad spectrum of diseases including cancer, cardiovascular, neurodegenerative, metabolic, infectious disease, and orphan genetic syndromes, which remain major therapeutic challenges. As detailed below, RNA-based drugs include several classes of molecules, such as messenger RNAs (mRNAs), which can be introduced into cells and then translated into target proteins for protein replacement therapy, supplementation, or vaccination; RNA aptamers which can directly bind to extracellular, cell surface, or intracellular proteins that are traditionally targeted by small molecules and protein-based drugs; Antisense oligonucleotides (ASOs), short interfering RNAs (siRNAs), and microRNAs (miRNA) that can be delivered into cells to target intracellular mRNA or functional ncRNAs through complementary base pairing, resulting in gene silencing or control of gene expression. These approaches allow interventions beyond the level of the genome, so that specific genes can be knocked out, mutations can be repaired, or even missing proteins can be replaced.

RNA-based drugs provide several advantages, especially their ability to target genetic components previously deemed “undruggable” by traditional small molecules or antibodies. Recent estimates suggest that only 2–5 % of disease-modifying proteins are likely to be druggable (Hopkins & Groom, 2002). In addition, numerous proteins share similarities in their structural composition, which poses a significant challenge in terms of target selectivity. RNA-based drugs, through Watson-Crick base pairing, enable highly specific gene targeting, reducing off-target effects and enhancing treatment efficacy. For instance, KRAS mutation G12D has been identified as a driving oncogene for pancreatic cancer development and is basically considered an undruggable target. The first RNAi designed to target this mutation is an anti-KRASG12D siRNA encapsulated in a biodegradable polymeric matrix (LOADER™). In an open-label phase I/IIa study (NCT01188785), the results indicated no dose-dependent toxicity, with the majority of pancreatic cancer patients displaying stable disease as well as a decrease of the tumor marker CA19–9 (Golan et al., 2015). Currently, anti-KRASG12D siRNA is under investigation in a Phase II study (NCT01676259) to further evaluate the therapeutic effectiveness in combination with chemotherapy. Another advantage of RNA-based drugs is their straightforward development process. Currently, the development of novel small molecules or recombinant proteins is both costly and time-consuming. In contrast, RNA-based drugs can offer a cost-effective solution and, once the target RNA sequence is established, the RNA-based drug can be rapidly designed and synthesized for clinical tests (Damase et al., 2021). This advantage is particularly important in the production of mRNA vaccines, allowing for a prompt and cost-effective response to possible pandemics (Kim, 2022). RNA-based drugs have also gained attention for their potential in treating genetic and rare diseases (Martini & Guey, 2019), which still represent notable therapeutic challenges. Rare diseases indeed present limited or no treatment options. In this respect, pharmaceutical companies may be reluctant to invest in research and development for therapies targeting these conditions, primarily due to the restricted patient population and the financial challenges associated with drug development. Recent advancements in RNA-based drugs, specifically RNAi and ASOs, have shown great potential in the treatment of rare diseases. ASOs and siRNAs can be customized to selectively silence disease-causing genes or modulate aberrant splicing events, providing targeted therapeutic interventions. For instance, in spinal muscular atrophy (SMA), ASOs targeting the survival motor neuron 2 (SMN2) gene have demonstrated remarkable efficacy in increasing SMN protein levels and improving disease progression (Darras et al., 2019; Finkel et al., 2017; Mercuri et al., 2018). siRNA and mRNA-based drugs offer another innovative approach, by directly delivering functional proteins to compensate for genetic deficiencies. This approach holds significant potential for rare diseases characterized by protein deficiencies, such as cystic fibrosis (Friedman et al., 2023) and hemophilia (Chen et al., 2020; Sehgal et al., 2015).

The versatility of RNA-based drugs makes them ideal candidates for personalized medicine approaches, providing the unique opportunity to address the underlying defects at a patient-specific level. In oncology, for instance, RNA-based drugs can target specific oncogenes or altered signaling pathways, enabling tailored treatments based on the molecular profile of a specific tumor (Morrow et al., 2012; Sarker et al., 2020). Moreover, ongoing clinical trials have demonstrated the potential for mRNA vaccines to be personalized and developed for each patient after RNA sequencing analysis of tumor tissue. These vaccines are designed to instruct the immune system of patients to elicit an immune response against specific cancer-associated neoantigens (Lang et al., 2022; Rojas et al., 2023).

Despite these significant advantages, substantial challenges remain, notably in delivery optimization, RNA stability, immunogenicity, and large-scale manufacturing. The primary drawbacks are associated with high molecular size and negative charge of RNA, which result in suboptimal pharmacokinetic and pharmacodynamic properties. Unmodified RNA-based drugs exhibit a short half-life due to scarce RNA stability and rapid degradation by endogenous nucleases in the intracellular space. Furthermore, efficient delivery of RNA-based drugs into target cells is hindered by several barriers, including the cell membrane, internalization processes, and most importantly, endosomal entrapment (Roth, 2005). Another significant limitation to overcome is the RNA chemical stability. Indeed, endogenous RNAs are highly sensitive to the three principal classes of intracellular RNA-degrading enzymes: endonucleases, which act by cleaving RNA at specific internal sites, 5′-

exonucleases, and 3′-exonucleases which hydrolyze RNA at the 5′ and 3′ end, respectively (Houseley & Tollervey, 2009). However, continued development in the area of lipid nanoparticle technology, conjugates, and other delivery systems is opening up better and safer therapeutics (Ciucci, Braga, & Zacchigna, 2025).

This review aims to explore the current state of RNA-based drugs, shedding light on the most significant breakthroughs, the challenges yet to overcome, and the potential impact at the pharmacological and therapeutic levels. More specifically, this review provides an updated, critical and insightful pharmacological analysis of both approved RNA-based drugs (Fig. 1) and the most relevant investigational RNA-based drugs discussing deeply pharmacological challenges, successes, failures, specific mechanisms of action (including the impact of drug delivery), tolerability and clinical aspects. Furthermore, this review uniquely explores the concept of RNA-based drugs repurposing, highlighting the opportunity to leverage shared pathophysiological mechanisms among different diseases to rapidly and cost-effectively expand therapeutic applications.

2. Classes of RNA-based drugs and their mechanisms of action

To date, different categories of RNA-based drugs have been developed and classified based on their structure and mechanism of action.

2.1. ASOs

Therapeutic ASOs consist of 18 to 30 base pairs of single-stranded synthetically designed RNA or DNA molecules composed of a phosphate backbone and sugar rings (Bennett et al., 2017). The ASOs' 3′ to 5′ sequence, through specific binding to the target pre-mRNA or mRNA, can act through two main mechanisms of action that either provoke the target RNA decay or inhibit processing steps (Fig. 2A). The most commonly used ASO mechanism is the recruitment of ribonuclease H (RNAse H) and subsequent target mRNA degradation (Wu et al., 2004). Human cells express two types of RNAse H: RNAse H1, active as a single peptide, and RNAse H2, which is a heterotrimeric enzyme (Crooke, 1999). ASOs are specifically designed to bind a target RNA sequence, usually in the mRNA coding region, thus forming a stable RNA:DNA hybrid structure that facilitates the recruitment of RNAse H1. RNAse H1 binds to the RNA:DNA heteroduplex through an RNA binding domain located on the N-terminus of the enzyme resulting in RNA cleavage and consequent downregulation of the target transcript (Vickers & Crooke, 2014). RNAse H1 is mainly located in the nucleus, where it can destroy maturing pre-mRNA and non-coding RNAs. RNAse H1 is also located in cytoplasm and mitochondria (Wu et al., 2004), where it degrades the mRNAs that escape the nucleus. ASOs can also act through an RNAse H1-independent mechanism by modulating alternative splicing, taking the name of "splice-switching" ASOs or splice-switching oligonucleotides (SSOs). The control of alternative splicing is primarily influenced by splicing factor proteins (Havens & Hastings, 2016), which can either promote (splicing enhancer) or inhibit (splicing silencer) splicing at specific sites. A splicing enhancer is a sequence element that, when bound by its corresponding protein, enhances the splicing of a nearby exon. Conversely, a splicing silencer is a sequence element that, upon binding by its corresponding protein, hinders or prevents splicing at a specific site. SSOs that base-pair with an enhancer introduce a steric block, preventing the stimulatory splicing factor from binding to its corresponding enhancer binding site. Consequently, this obstruction disrupts the splicing process, resulting in exon skipping. In contrast, an SSO that base-pairs with a splicing silencer sequence interferes with its silencing activity by blocking the binding of a negatively acting splicing factor. This action activates splicing sites that are normally suppressed by the silencer element, leading to the inclusion of the exon. These ASOs can be used to correct splicing but also to inactivate proteins by skipping specific exons (Mout et al., 2017; Siwkowski et al., 2004; Wancewicz et al., 2010). Interestingly, SSO nucleotides are designed to prevent the recruitment of the RNA-cleaving enzyme RNAse H in order to avoid the degradation of the pre-mRNA-SSO complex (Rigo, Seth, & Bennett, 2014). The straightforward design and synthesis of ASOs allow for relatively quick progression from concept to clinical use, which is especially valuable in precision and personalized medicine (Gagliardi & Ashizawa, 2021). ASOs can be administered via several routes, including intravenous, subcutaneous, topical, and intrathecal delivery, making them adaptable to different medical needs. Compared to other drug classes, ASOs are generally well-tolerated by the immune system, reducing the risk of severe adverse reactions (Gagliardi & Ashizawa, 2021). However, efficient delivery to target tissues, especially across biological barriers like the blood-brain barrier (BBB), represents a significant limitation. Poor cellular uptake often requires direct administration methods (e.g., intrathecal delivery for central nervous system diseases). Moreover, ASOs cannot be used for the treatment of all genetic disorders. This depends on the pathophysiology of the specific disease and the type of the genetic mutation (Lauffer et al., 2024).

2.2. mRNAs and mRNA vaccines

Therapeutic protein-coding mRNAs are synthetic molecules specifically designed to have a similar structure to endogenous mature and processed mRNA in the cytoplasm of eukaryotic cells (Kormann et al., 2011). Structurally, synthetic mRNAs are single-stranded molecules that

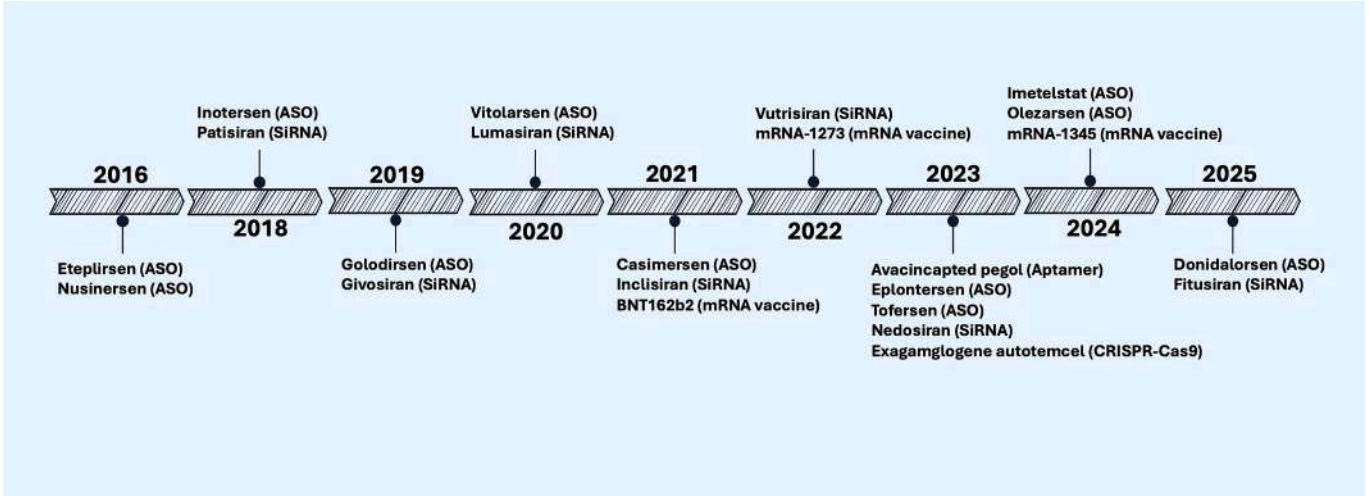

Fig. 1. Timeline of FDA-approved and commercially available RNA-based drugs.

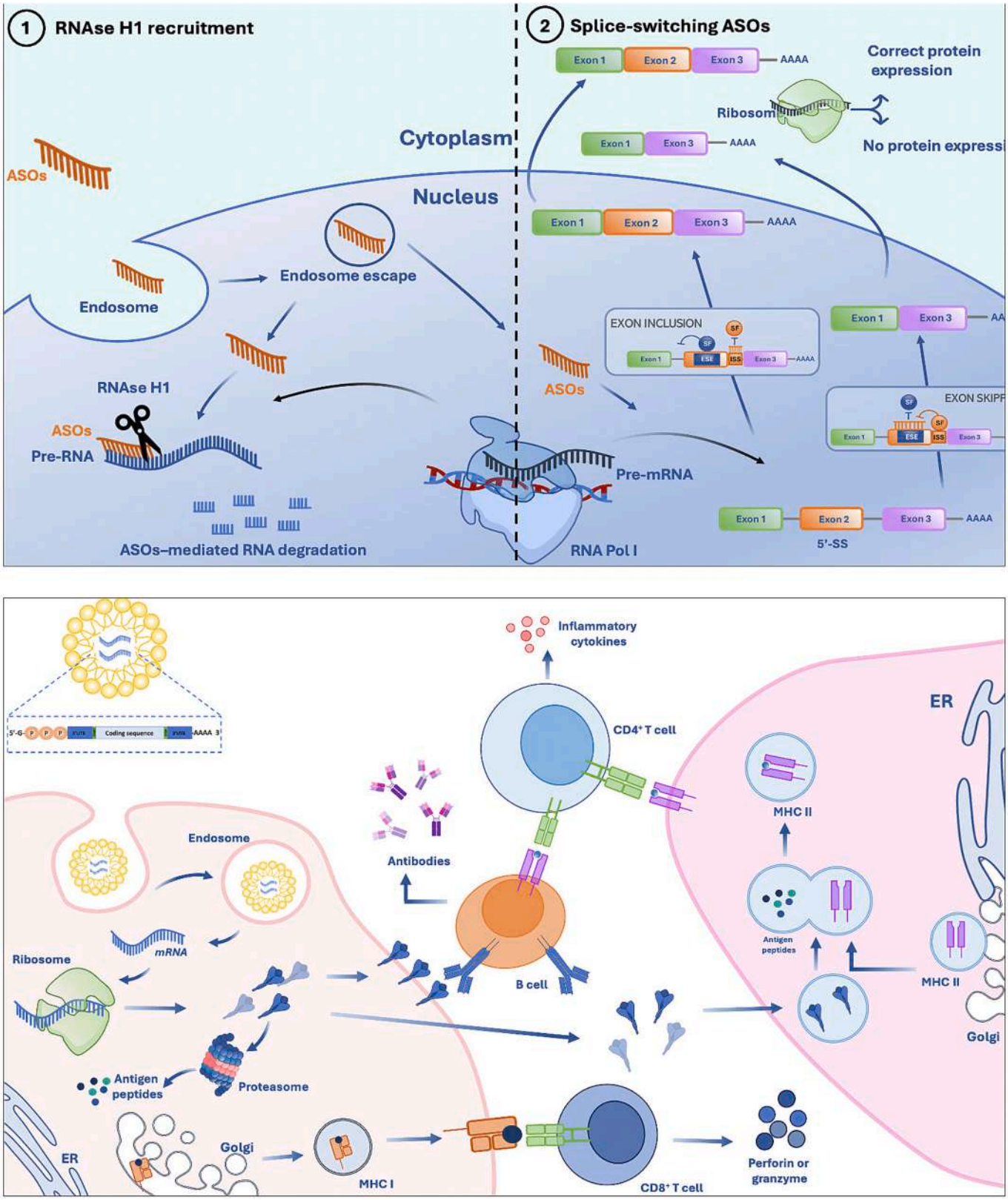

Fig. 2. Mechanism of action of ASOs and mRNA vaccines.
A. The RNAse H1-mediated mechanism involves the hybridization of ASOs with target RNA to form an RNA-DNA duplex, which is subsequently recognized by RNase H1. This enzyme cleaves the RNA strand, resulting in RNA degradation and a consequent reduction in gene expression. In the splice-Switching mechanism, ASOs bind to specific pre-mRNA splice sites or regulatory elements, thereby blocking the access to the spliceosome. Through this mechanism ASOs modulates the splicing process, leading to exon skipping, exon inclusion, or alternative splicing to restore or modify protein functions.

feature a 5'cap and a 3'poly(A) tail for ribosome-mediated translation and stability, and an open reading frame that encodes for the gene of interest, marked by start and stop codons, and flanked by a 5'and 3′ untranslated regions. Basically, mRNA-based drugs introduce synthetic mRNA into the cells, which subsequently directs the production of specific proteins for protein replacement therapy and vaccination. Once the mRNA enters the cytoplasm, its pharmacological activity is regulated by the same complex cellular machinery responsible for the translation of endogenous mRNA. The encoded protein product represents the pharmacologically active molecule, and its destination is established by signal peptides, whether they are natural proteins or recombinant engineered sequences, which guide the protein to the target cell compartment (Owji et al., 2018). mRNA-based drugs exhibit high effectiveness in delivering genetic information into the cells, leading to robust protein production without the need for nuclear entry, unlike DNA-based therapies (Qin et al., 2022). In addition, mRNA platforms allow for tailored therapies by considering patient-specific factors such as age, gender, and disease state to maximize efficacy and safety (Shi et al., 2024). However, it is important to underscore that challenges exist related to molecular instability, delivery, immune activation, and individual variability to mRNA-based drugs (Eftekhari et al., 2024).

In contrast to viral vector or DNA-based vaccines, mRNA vaccines are specifically engineered to facilitate the rapid expression of the encoded antigen, thereby eliciting a rapid and robust immune response (Chaudhary, Weissman, & Whitehead, 2021). After administration, the mRNA is taken up by antigen-presenting cells (APCs), such as dendritic cells, where it is recognized by ribosomes and translated into antigenic protein utilizing the same cellular machinery responsible for the translation of endogenous mRNA (Fig. 2B). The newly synthesized antigen is subsequently processed by the proteasome complex into smaller fragments in the cytosol. This process exposes the antigenic epitopes, which form complexes with Major Histocompatibility Complex Class I (MHC I) molecules and presented on the cell surface to CD8+ T cells. Consequently, CD8+ T cells release cytotoxic molecules, including perforin and granzyme, which induce apoptosis in infected or antigen-expressing cells (Lazzaro et al., 2015). Simultaneously, some extracellular or endosomal antigen fragments are processed through the Major Histocompatibility Complex Class II (MHC II) pathway and recognized by MHC II molecules, resulting in the activation of T helper cells expressing CD4 (Oberli et al., 2017). The response of T helper cells can be enhanced by introducing a secretion signal into the antigen-encoding sequence, thereby redirecting the protein antigen to the extracellular space. Depending on the cytokine environment, CD4+ T cells can differentiate into various subtypes. Th1 cells primarily secrete interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which activate macrophages and support cellular immunity. In contrast, Th2 cells secrete interleukins such as IL-4, IL-5, and IL-13, which facilitate the production of specific antibodies, the activation of eosinophils and basophils, and the promotion of inflammatory response. Furthermore, CD4+ T cells also play a pivotal role in supporting B cell maturation and differentiation into plasma cells, which are responsible for producing antigen-specific antibodies (Shi et al., 2024).

Unlike traditional vaccines that require growing live viruses, mRNA vaccines use synthetic genetic code, enabling much faster design and manufacturing (Pardi et al., 2018). This rapid adaptability was crucial during the COVID-19 pandemic and allows quick updates to target emerging viral variants or different pathogens. mRNA vaccines are non-infectious and non-integrating, eliminating risks of infection or insertional mutagenesis. The mRNA is naturally degraded by cellular processes, and its immunogenicity can be modulated by chemical modifications to improve safety. In summary, mRNA vaccines represent a transformative advance in vaccinology, combining high efficacy, safety, and adaptability with rapid development capabilities (Pardi et al., 2018). However, challenges remain in stability, delivery, storage, and unbiased global access. Ongoing research aims to improve formulations, extend shelf life, reduce side effects, and broaden applications beyond infectious diseases to cancer and other therapeutic areas.

2.3. RNA interference: siRNA and miRNA

RNA interference is an RNA-dependent conserved mechanism involving the regulation of gene expression through the inhibition of mRNA activity mediated by double-stranded RNA (Fire et al., 1998). This process begins with the introduction of double-stranded RNA into the cell and is guided by siRNAs, typically released by exogenous sources such as synthetic siRNA or viral infections, or endogenous pre-miRNA transcribed from genomic DNA and processed by the enzyme Drosha in the nucleus in transcripts with an internal loop or short hairpins structure (Fig. 3A). Both exogenous or endogenous double-stranded RNAs are cleaved by the cytoplasmatic RNAse III enzyme, Dicer, into short double-stranded mature siRNAs fragments of 20–25 base pair or miRNAs, respectively (Ketting et al., 2001). Subsequently, siRNA fragments are incorporated into a premature RNAi-induced silencing complex (pre-RISC), a ribonucleoprotein complex that includes siRNA or miRNA and an Argonaute (Ago2) protein, which represents the catalytic component of RISC. The maturation from pre-RISC to RISC is mediated by an ATP-dependent RNA helicase that unwinds the double-stranded RNA generating two single-stranded siRNA (Nykanen, Haley, & Zamore, 2001). The sense strand, also called "passenger", is rapidly degraded by exonuclease. By contrast, the antisense strand is retained as a functional component in the mature RISC, serving as a guide for the protein complex to the target mRNA sequence (Birmingham et al., 2007). RISC uses the single-stranded guide RNA to find the target mRNA sequence in the cytosol. The complementary binding of the guide strand to the target mRNA triggers the slicer endonuclease activity of Ago2 protein to cleave the phosphodiester bond in the target mRNA backbone (Liu et al., 2004), resulting in two mRNAs fragments, one without the 5'cap and the other lacking the poly(A) tail, which are rapidly degraded by RNAses. siRNAs and miRNAs have comparable physicochemical characteristics but different functions. Both molecules are short double stranded RNAs involved in post-translational gene regulation, but they differ in the mechanism of action. The primary function of siRNAs is to induce the degradation of specific target mRNAs. siRNAs typically have perfectly complementary base-pairing to the target mRNA resulting in gene silencing without off-target effects (Lam et al., 2015).

miRNAs (Fig. 3A) conversely are involved in the regulation of a wide range of physiological processes, including development, cell differentiation, and immune response, and typically target multiple mRNAs. miRNAs tend to have incomplete or imperfect base-pairing, usually occurring at the seed region (nt2–7 of the 5′ end) that results in translation repression or mRNA destabilization without mRNA degradation (Djuranovic, Nahvi, & Green, 2012). Interestingly, miRNA therapeutic application relies on two different strategies: inhibition and replacement. The first strategy aims to inhibit endogenous miRNAs by the use of miRNA antagonists, also known as antagomiRs or anti-miRs. These antagonists are designed to have enhanced affinity and trap the endogenous target miRNA in a conformation that cannot be processed by RISC, or otherwise, causes miRNA degradation (Kong et al., 2012). On the other hand, the miRNA replacement approach consists of a synthetic miRNA, also called miRNA mimics, administered to mimic the functioning of endogenous miRNAs, i.e. tumor suppressor miRNA to restore a loss-of-function mutation (Mollaei, Safaralizadeh, & Rostami, 2019). The design of RNA interference as therapeutic agents must ensure the complementarity of the antisense "guide" with the target mRNA sequence to maximize the selectivity and potency. Moreover, it is crucial to synthesize strands that avoid off-target binding to homologous mRNA sequences; even a 7-base complementary domain can activate RISC (Setten, Rossi, & Han, 2019). Both siRNA- and miRNA-based drugs can be used for therapeutic approaches depending on gene silencing but have distinct advantages and limitations (Lam et al., 2015). siRNA-based drugs, by targeting a single mRNA, are highly selective. This is advantageous because minimizes off-target gene silencing. Several siRNA-

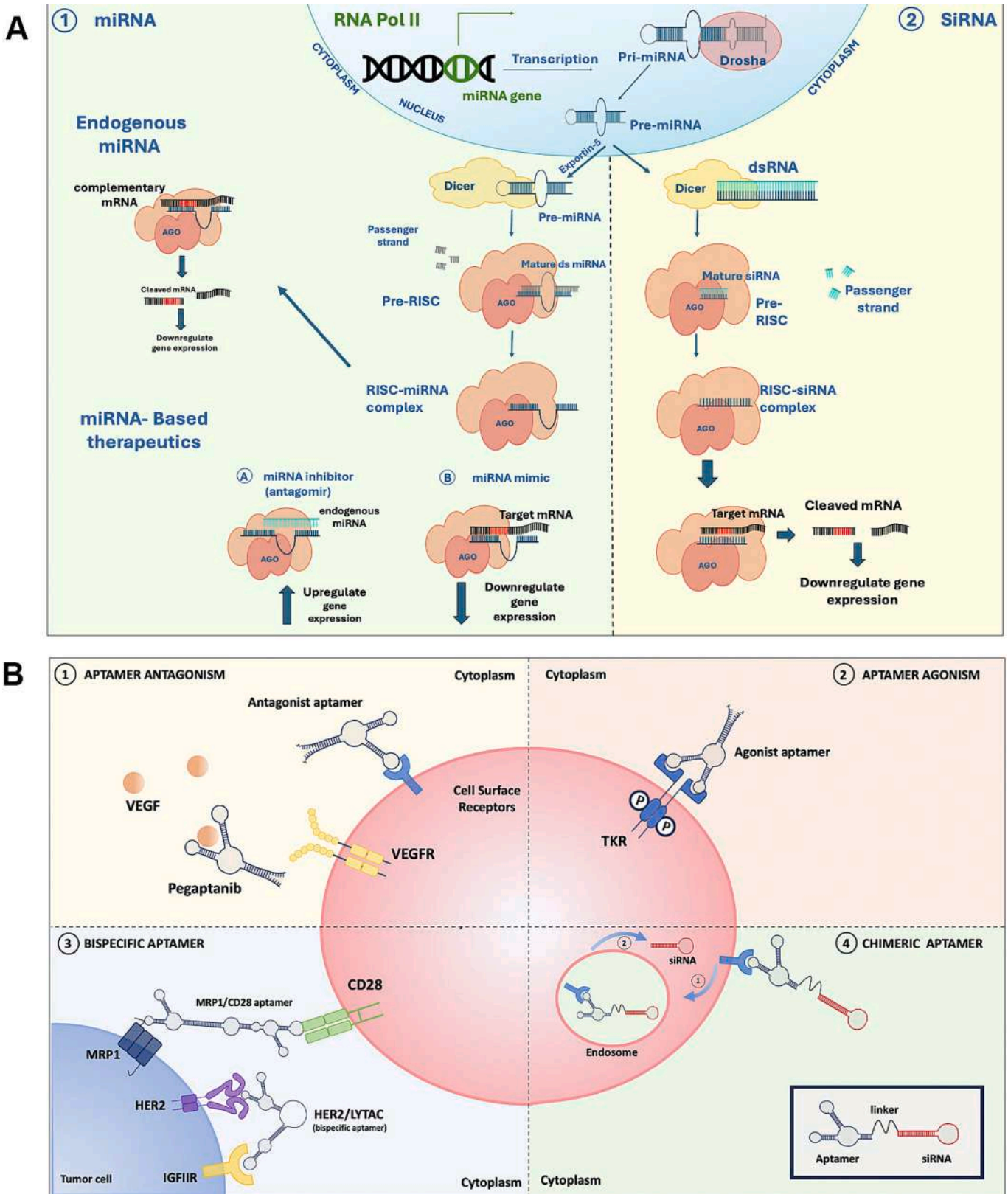

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Fig. 3. Mechanism of action of Aptamers and RNAi.
A. Illustration representing the biosynthesis and the mechanism of action of RNAi.
1. miRNA genes are transcribed by RNA polymerase II into a primary miRNA (pri-miRNA), which is subsequently cleaved by the enzyme Drosha, yielding a ~ 70 nucleotide precursor miRNA (pre-miRNA) characterized by a hairpin structure. Pre-miRNA is transported into the cytoplasm by Exportin-5, where it is further processed by Dicer, resulting in a ~ 22 nucleotide double-stranded miRNA. The guide strand is incorporated into the RISC,while the passenger strand is degraded. The guide strand within RISC binds to target mRNA, primarily in the 3' UTR. miRNA can downregulate or block mRNA translation, mimicking the action of endogenous miRNA or functioning as a miRNA inhibitor or antagomir by binding to a target miRNA and preventing its interaction with the mRNA target.
2. siRNA molecules can be synthetic or endogenously generated from longer double-stranded RNA processed by Dicer. siRNAs are then loaded into the RISC protein complex, where the passenger strand is removed and degraded, while the guide strand remains associated with RISC, guiding it to complementary mRNA sequences in the cytoplasm. RISC cleaves the target mRNA precisely at the site corresponding to the siRNA sequence, resulting in mRNA degradation and subsequent gene silencing.
B. Schematic illustration of aptamers the mechanisms of action. 1. Aptamer Antagonism: Aptamers can bind to the active or binding site of a target protein, thereby preventing its interaction with its natural ligand or receptor. For example, pegaptanib, an FDA-approved aptamer utilized in the treatment of age-related macular degeneration, functions as an antagonist by blocking VEGF signaling. 2. Aptamer Agonism: Aptamers can also mimic endogenous ligands or induce receptor dimerization of tyrosine kinase receptors, thereby stimulating downstream signaling even in the absence of the natural ligand. 3. Bispecific Aptamers: these are single engineered aptamer molecules capable of simultaneously binding to two different targets. The HER2/LYTAC bispecific aptamer is a molecule that combines an insulin-like growth factor 2 receptor (IGFIIR)-binding DNA aptamer and a HER2-binding DNA aptamer, enabling it to bind to both HER2 and IGFIIR. 4. Chimeric Aptamers: These are engineered hybrid molecules that integrate two or more different functional elements into a single aptamer. For instance, an aptamer conjugated with a non-aptamer moiety, such as siRNA, allows the aptamer to guide siRNA specifically to cancer cells, facilitating selective gene silencing.

based drugs have been approved, demonstrating clinical translatability. siRNA-based drugs can however show poor stability in vivo due to degradation by nucleases, leading to short half-life and poor pharmacokinetics (Hu et al., 2020). By contrast, miRNA-based drugs can target numerous mRNAs simultaneously, modulating entire gene networks and pathways. This is useful for treating complex multigenic diseases like cancers and neurodegenerative disorders, where multiple genes/pathways are dysregulated. However, these multi-target mechanisms increase the risk of off-target effects impacting physiological pathways (Lam et al., 2015).

2.4. Aptamers

Aptamers are short synthetic single-stranded oligonucleotides or peptide sequences able to specifically and tightly bind a target (e.g., proteins, small molecules, ions, or cells (Fig. 3B). The mechanism of action of aptamers is basically determined by their ability to mimic antibodies, which has led to their classification as "chemical antibodies" or "antibody mimics" (Keefe, Pai, & Ellington, 2010). In contrast to traditional antibodies, aptamers exhibit peculiar properties such as enhanced stability, reduced immunogenicity and lower molecular weight, which facilitate the delivery to the target site and promote rapid plasma clearance (Thiviyanathan & Gorenstein, 2012). Following binding to a specific target, aptamers mostly exert inhibitory effects by blocking protein-protein interactions, such as receptor-ligand interactions, thereby acting as antagonists. Indeed, whereas most aptamers act as antagonists, some are agonists or allosteric modulators. For example, certain aptamers can promote oligomerization of their target proteins or enhance enzymatic activity, depending on their binding site and the conformational changes they induce (Keefe et al., 2010). Aptamers can also be engineered to act inside cells and in this case are referred as "intramers." These intracellular aptamers can bind to and modulate the activity of proteins within the cytoplasm or nucleus, affecting processes such as gene expression, RNA transport, or enzymatic activity (Yoon & Rossi, 2018). Bispecific aptamers are engineered nucleic acid molecules designed to simultaneously bind two different targets, typically a tumor-associated antigen and an immune cell receptor. This dual specificity allows them to physically bridge immune effector cells (such as T cells or NK cells) with tumor cells, thereby enhancing immune-mediated tumor cell killing (Thomas, Porciani, & Burke, 2022). Interestingly, chimeric aptamers are engineered nucleic acid molecules composed of an aptamer linked to another functional entity, which can be another aptamer, a therapeutic nucleic acid (such as siRNA or miRNA), a peptide, a protein, or even a nanoparticle. This type of design enables chimeric aptamers to combine the high specificity and affinity of aptamers for their targets with additional functionalities, such as targeted drug delivery and gene silencing (Kanwar, Roy, & Kanwar, 2011).

The identification and screening of designed aptamers originate from SELEX (Systematic Evolution of Ligands by Exponential Enrichment), an in vitro process that combines combinatorial chemistry techniques with molecular biology (Ellington & Szostak, 1990). The screening of aptamers via SELEX consists of three main steps, which are repeated for six to ten cycles using randomized oligonucleotide sequences that are successively amplified to form a library through enzymatic activities (Ellington & Szostak, 1990). Individual members of the selected library are then identified and sequenced. This process allows for the generation of aptamers with high binding affinity and specificity to precise targets. Aptamers play key roles thus in a variety of applications including biosensing, bioimaging, drug delivery, therapeutics and diagnostics (Keefe et al., 2010; Kim, Raston, & Gu, 2016; Zhang, Lai, & Juhas, 2019). Aptamers exhibit however limitations such as vulnerability to degradation by nucleases in the bloodstream, resulting in short half-lives and limiting their therapeutic window unless chemically modified (Gao et al., 2022).

2.5. Other therapeutically relevant RNAs

This section briefly introduces certain classes of RNA-based therapeutics, such as Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated (CRISPR-Cas) system, guide RNAs (gRNAs), transfer RNAs (tRNAs), circular RNAs (circRNAs) and ribozymes, which are included here to provide a comprehensive overview of RNA therapeutic landscape. Although these RNA-based approaches show promising therapeutic potential (Baptista et al., 2021), they are not the main focus of this review and are therefore briefly discussed.

2.5.1. CRISPR-Cas system and gRNAs

The CRISPR-Cas system is a revolutionary gene-editing technology originally derived from a bacterial adaptive immune mechanism, which defends against viruses and plasmids by targeting and cleaving foreign nucleic acids (Menon et al., 2025). CRISPR/Cas systems are classified into two main categories: Class I, which include type I, III, and IV, consists of a CRISPR locus accompanied by multiple Cas proteins that form a multi-subunit complex for target recognition and cleavage; Class II, which encompasses type II, V, and VI, in which the system relies on a single, large Cas protein, such as Cas9, Cas12, or Cas13, to perform both target recognition and cleavage (Xu & Li, 2020). The functionality of the CRISPR-Cas system depends on the utilization of engineered gRNAs and RNA-guided Cas nucleases (Wiles et al., 2015). The gRNA is specifically designed to base-pair with a target sequence and binds to a Cas protein, thereby forming a Cas-gRNA ribonucleoprotein complex. This complex recognizes a short DNA motif known as the protospacer-adjacent motif and a 20-nucleotide sequence that is complementary to the gRNA

(Doudna & Charpentier, 2014). Upon target recognition, the Cas nuclease cleaves the double stranded DNA, or in certain systems, single stranded RNA, at the specific target site, thus facilitating precise genome editing. Unlike traditional small molecule or antibody drugs, CRISPR-Cas-based drugs exploit the precision of gRNAs to guide gene-editing enzymes to specific DNA sequences, allowing for highly targeted modifications. The ability to precisely edit genes opens up a vast array of therapeutic possibilities for various diseases including genetic disorders, cancer, infectious diseases, neurological disorders and ophthalmic diseases. Despite the immense promise, several challenges related to delivery, off-target effects, immunogenicity and editing efficiency need to be addressed for widespread clinical application of CRISPR-Cas-based drugs.

2.5.2. tRNAs

tRNAs are small molecules, typically 70–90 nucleotides in length, which play a critical role in translating mRNA codons into their corresponding amino acid during protein synthesis. Each tRNA exhibits a characteristic cloverleaf-like secondary structure, which subsequently folds into a more compact L-shaped tertiary structure. A specific amino acid is covalently attached to the 3′ acceptor stem of the tRNA, while the anticodon loop contains a three-nucleotide sequence, known as the anticodon, which specifically recognizes and pairs with a complementary codon on the mRNA molecule. tRNA-based drugs represent a rapidly emerging class of RNA therapeutics designed to treat genetic diseases at the level of protein translation (Ward et al., 2024). These therapies leverage engineered tRNAs to address mutations that disrupt normal protein synthesis, particularly nonsense and missense mutations. Engineered tRNAs, known as suppressor tRNAs, can recognize premature stop codons (nonsense mutations) in mRNA and insert an amino acid, allowing the ribosome to produce a full-length, functional protein (Albers et al., 2023). Moreover, these tRNAs can insert the correct amino acid at sites of missense mutations, potentially restoring protein function. tRNA drugs are being explored for a variety of genetic diseases, including cystic fibrosis, muscular dystrophy, genetic epilepsies (e.g., Dravet syndrome), liver-based genetic disorders and other rare and ultra-rare diseases caused by nonsense or missense mutations. Suppressor tRNAs can be designed to target only the mutated stop codon, reducing off-target effects compared to small-molecule readthrough drugs like ataluren (Ward et al., 2024). Engineered suppressor tRNAs have restored functional protein expression in preclinical models of diseases like cystic fibrosis (Albers et al., 2023). tRNA-based drugs hold significant advantages. A single engineered tRNA designed to read through a specific premature stop codon can potentially treat thousands of different genetic diseases caused by that same mutation, regardless of the specific gene affected. Unlike some other genetic therapies, tRNA-based drugs aim to restore the production of the complete, functional protein, which is often crucial for therapeutic efficacy (Coller & Ignatova, 2024). However, challenges related to efficient cellular delivery and stability remain critical for clinical translation.

2.5.3. CircRNAs

CircRNAs are a class of endogenous, covalently closed RNA molecules that have garnered significant attention due to their stability, tissue- and cell-specific expression patterns, and different regulatory roles in gene expression. In contrast to linear RNAs, circRNAs are characterized by the absence of free 5′ and 3′ ends, which confers resistance to exonuclease-mediated degradation, thereby enhancing their stability (Liu & Chen, 2022). circRNAs play a role in several physiological and regulatory functions, including modulation of protein translation and regulation of both protein and RNA activity. These intrinsic properties make circRNAs promising candidates for engineering of synthetic molecules designed to modulate specific targets, thereby facilitating a wide range of therapeutic applications. circRNAs functions through diverse mechanisms of action, ranging from miRNA sponging to direct interaction with RNA and protein targets. Indeed, one of the most well-characterized mechanisms of circRNAs is miRNA sponging (Schreiner et al., 2020). CircRNAs can contain multiple binding sites for specific miRNAs, allowing them to sequester miRNAs in a sequence-dependent manner and inactivate their functions, thus preventing the inhibition of their mRNA targets (Sakshi et al., 2021). This mechanism can be strategically redirected to any miRNA using specifically designed circRNAs engineered with tailored miRNA-binding sites. In addition to miRNA sponging, circRNAs can be designed to target and interact directly with pre-mRNA and mRNAs transcripts in a sequence-dependent manner. Such interactions can lead to alternative splicing by competing with splicing factors or influencing splice site selection, as well as affecting mRNA translation through steric hindrance or alterations in RNA secondary structures that influence translation efficiency (Ren et al., 2023). Moreover, engineered circRNAs may be used to disrupt pathogenic or defective RNA secondary structures through competitive annealing, thereby restoring normal RNA function or inhibiting the translation of aberrant proteins (O'Leary et al., 2025). The protein-coding potential of circRNAs suggests promising applications in the development of vaccines for infectious disease, cancer immunotherapies, and protein replacement therapies (Fan et al., 2022). In addition to the direct regulation of RNA functions, circRNAs also interact with RNA-binding proteins to influence their localization, activity, or availability. Through binding to RNA-binding proteins, such as Protein Kinase R (PKR) or heterogeneous nuclear ribonucleoprotein L (HNRNPL), via a mechanism analogous to miRNA sponging, circRNAs can modulate protein activity or interactions, effectively inactivating protein functions within cells and inhibiting their disease-related functions (Das et al., 2021). CircRNA therapeutics represent a promising next generation of RNA-based drugs with advantages in stability, protein expression, and immunogenicity. Although no circRNA drugs have yet been approved by the FDA, the field is rapidly advancing. Continued improvements in manufacturing, delivery, and clinical evaluation are expected to drive circRNA drugs toward regulatory approval within the next few years.

2.5.4. Ribozymes

Ribozymes represent a promising class of RNA-based drugs with ongoing advances. Ribozymes, or RNA enzymes, are small RNA molecules endowed with intrinsic enzymatic activity, able to recognize and bind specific RNA sequences, thereby catalyzing reactions such as the cleavage of deleterious RNA or the repair of mutated RNA. In contrast to protein enzymes, ribozymes catalyze biochemical reactions exclusively through their RNA structure, which facilitates the regulation of gene expression at the post-transcriptional level. Their sequence-specific binding and catalytic functions confer significant potential for therapeutic applications, particularly in the degradation of pathogenic RNAs and the correction of genetic mutations at the RNA level. Through complementary base-pairing interactions, ribozymes can selectively bind target mRNAs via dedicated recognition domains. This hybridization fosters precise interactions with cytoplasmic mRNAs, enabling site-specific cleavage or modulation of transcript stability and translation (Bird et al., 2005). The catalytic activity of ribozymes is mediated by a conserved structural domain responsible for the hydrolysis of phosphodiester bonds within the RNA backbone. This reaction, typically characterized as a self-catalyzed transesterification, results in the cleavage of the target mRNA into two fragments: one bearing a 2′,3′-cyclic phosphate and the other a 5′- hydroxyl group. Such cleavage leads to transcript degradation and effectively abrogates downstream protein synthesis (Maurel, Leclerc, & Herve, 2020). In addition to their catalytic degradation function, ribozymes may also inhibit gene expression by sterically obstructing ribosomal access to the mRNA, thereby preventing translation initiation or elongation. This antisense-like mechanism does not require catalytic activity but instead leverages the ribozyme's sequence specificity and stable hybridization (Serganov & Patel, 2007). Although clinical success has been limited so far, new ribozyme designs and delivery strategies are actively being developed to overcome current

challenges.

3. Challenges in the discovery, development and delivery of RNA-based drugs

Despite the important therapeutic potential, the progress of RNA-based innovative pharmacological treatments encounter several challenges that require resolution to facilitate broader applications. Although RNA-based drugs can potentially target genetic components that are "undruggable" using small molecules or antibodies, the identification of druggable RNA targets is a challenging process that needs extensive investigations (Yu, Choi, & Tu, 2020). It is firstly crucial to understand if a specific RNA target under investigation can be reached by RNA-based molecules. This can depend on the quantity of the RNA target within the cells among complex cellular substances, and also on the binding to highly structured and functional binding sites. It is also important to understand whether the pharmacodynamic interaction between a RNA-based drug and a RNA target is functional and sufficient for the induction of the pursued pharmacological effect. The assessment of the specificity of the binding is another key point to be assessed in line with the classical process of drug development. In this regard, a potential problem is related to the fact that both RNAs and DNAs are negatively charged nucleic acids having shared structural features. To overcome this limitation, structural modifications and optimizations are essential to increase the selectivity toward RNA targets (Costales et al., 2020).

The cleavage of RNAs catalyzed by RNA-degrading enzymes represents another major limitation. In this respect, recent advancement has been made to improve the stability of RNA, including the introduction of chemical modifications into bases (Bost et al., 2021), RNA backbones (Ayadi et al., 2019), or sugar moieties (Gangopadhyay & Gore, 2022).

In contrast to small molecules that are specifically designed and developed to have a limited size, no charged atoms, and are characterized by moderate lipophilicity, the vast majority of RNA-based drugs are highly negatively charged, hydrophilic macromolecules that have scarce or no ability to passively cross the lipid bilayer cell membrane. The uptake into the cells is mediated by endosomes, which similar to the cell membrane, contain a lipid bilayer that entraps most of the administered RNA-based drug (Dowdy, 2017). To overcome this limitation, many delivery systems have been developed, allowing effective delivery without extensive structural modifications, such as the addition of targeting moieties and the encapsulation of RNAs into lipid or polymeric nanoparticles. The conjugation process involves the covalent attachment of RNA-based drugs with compounds recognized by specific cell surface receptors. It is reported that this method improves cell or tissue-specific delivery through receptor-mediated endocytosis. Specific cases are the *N*-acetylgalactosamine (GalNAc)-conjugated siRNAs, givosiran, and inclisiran, in which the GalNAc moiety is attached to the 3′ end of the passenger strand of the siRNA. The GalNAc moiety is recognized by the asialoglycoprotein (ASGPR) receptor, a protein abundantly found on hepatocytes, resulting in targeted delivery to the liver (Springer & Dowdy, 2018). However, no other specific conjugate for cell-specific delivery has been approved following clinical studies. The successful targeting of liver cells through GalNAc conjugation takes advantage of the high expression of ASGPR uniquely on hepatocytes. Attempts to replicate this approach in non-hepatic tissues have been limited primarily by the lack of receptors with similar abundance and internalization efficiency. For instance, the targeting of the muscle tissue is challenged by poor accessibility and lack of specific receptors for ligand-mediated delivery. In this regard, various peptides and antibodies targeting transferrin receptor 1 or integrins have been explored. However, these approaches have shown limited clinical success because of issues with tissue penetration, specificity, and systemic side effects (Chen et al., 2025). As aforementioned, in the central nervous system (CNS) the presence of the BBB represents a major challenge for the delivery of RNA-based drugs. Various receptor-mediated transcytosis pathways, including those involving transferrin, insulin, and low-density lipoprotein receptor-related protein 1, have been investigated (Chen et al., 2025). However, delivery efficiency remains limited due to receptor saturation, restricted penetration into brain tissues, and potential immunogenicity. Regarding immune cells, targeting strategies often involve ligands for surface markers like CD4, CD8, or Toll-like receptors. Nevertheless, the heterogeneous expression of these receptors and the dynamic nature of immune cells (Satija & Shalek, 2014) complicate effective and specific delivery, with safety concerns related to off-target immune activation and toxicity.

Other gene delivery methodologies include the use of viral vectors, such as retrovirus or lentivirus, that can be engineered as gene-delivery recombinant vehicles by changing part of the virus genome with therapeutic agents (Bulcha et al., 2021). The major advantage of the use of viral vectors is their ability to protect from biological degradation and to efficiently cross cellular barriers. Although recombinant viruses are designed to be non-pathogenic, there exist the possibility of unwanted severe immune responses, inflammatory reactions, or mutagenesis due to the insertion of exogenous DNA into the host genome. To overcome these challenges other non-viral delivery carriers have been developed. Encapsulation of RNA into lipid-based nanoparticle (LNPs) or polymer-based nanoparticles (PNPs) has been shown to enhance the delivery of RNA-based drugs and protect them against nuclease-mediated degradation. LNP drug-delivery formulations are the only FDA-approved non-viral nucleic acid delivery system capable of delivering large payloads (Yin et al., 2014). LNP action involves uptake in endosomes, in which the interaction between the cationic lipids of the LNPs and the anionic lipid of the membrane disrupts the membrane structure resulting in dissolution of the LNP and in the cytosolic release of the oligonucleotides (Schlich et al., 2021). Whereas clinically validated for efficiency and biocompatibility, it has been reported that cationic lipids in LNPs are associated with inflammatory responses, complement activation, and potential liver toxicity (Jung et al., 2022). This can limit dosing regimens and raise immunogenicity concerns. To address these limitations, next-generation nanoparticles have been developed. These nanoparticles are based on new, non-lipid materials such as fusogenic proteins (Zeng et al., 2023) or polymers (Yang et al., 2023). In this regard, PNPs are currently investigated as a promising tool to improve the bioavailability and/or specific delivery of RNA-based drugs. PNPs indeed offer several advantages, such as including improved biocompatibility, reduced immunogenicity, and adjustable physicochemical properties that enable controlled release and lower toxicity in comparison with LNPs (Niculescu, Birca, & Grumezescu, 2021). However, scaling up the manufacturing of PNP presents significant challenges including maintaining batch-to-batch reproducibility, controlling the distribution of particle size, and meeting stringent regulatory quality standards (Desai, 2012).

Interestingly, novel protein-based delivery systems have gathered significant attention for RNA delivery. These systems can potentially address specific issues including liver accumulation and immunogenicity, which are often associated with the use of nanoparticle-based systems (Zhu et al., 2023). Moreover, protein-based delivery systems have significant advantages compared to other delivery systems. For instance, the preparation processes of protein carriers are straightforward and consequently cost-effective in large-scale production and purification. Among protein-based delivery systems, the use of antibodies for RNA delivery holds great promise for advancing therapeutic strategies. In 2015, the first direct conjugation of antibodies to siRNA was achieved by creating the siRNA delivery platform THIOMABs (Cuellar et al., 2015), which identified antibodies capable to covalently bind chemically stable siRNA. The antibody-mediated RNA delivery technology took a step forward with the development of Antibody-Oligonucleotide Conjugate (AOC) technology (Qian et al., 2023). In comparison with the typical oligonucleotide therapy, AOC technology exhibits better pharmacokinetic properties as well as more precise biodistribution. Furthermore, AOC technology avoids the toxic side effects

observed with cationic lipids in LNPs and benefit from established, scalable antibody production methods (Meng et al., 2025). The conjugation process is versatile considering that several types of oligonucleotides, such as siRNA and ASO can be conjugated to AOC. The conjugation stability and efficiency however still require optimization for clinical application.

It is reasonable to claim that among plenty of RNA delivery systems developed, protein-based delivery systems can significantly advance the therapeutic value of RNA-based drugs. However, to promote the protein-based RNA delivery systems toward the clinical application, further efforts are needed to overcome existing issues of this technology. This comprises for instance the establishment of more efficient delivery methods to improve oral bioavailability and other technical interventions aimed at extending drug half-life.

4. Clinical applications and pharmacological aspects of RNA-based drugs

4.1. Approved ASO-based drugs

To date, fourteen ASO-based drugs have been approved, addressing a range of rare genetic and metabolic diseases, with ongoing approvals expanding their therapeutic value. These drugs are significantly changing treatment paradigms, especially for conditions with limited treatments, and represent a growing therapeutic class together with siRNA and aptamer drugs.

Fomivirsen (Ionis/Novartis) is the first ASO ever approved by FDA in 1998 for the local treatment of cytomegalovirus (CMV)-induced retinitis in patients with acquired immunodeficiency syndrome (AIDS) (Perry & Balfour, 1999). It was also approved in the European Union (EU) in 1999. Fomivirsen exerts its therapeutic effects by binding to the complementary sequence of the mRNA transcripts of the major immediate early region 2 (IE2) of human CMV. This inhibits the translation of the IE2 proteins (specifically the 86 kDa and 55 kDa isoforms), which are essential for viral gene expression and replication of infectious CMV. Despite being a pioneering drug, fomivirsen was discontinued in the U.S. in 2006 (and in the EU in 2002) because the success of antiretroviral therapy in the early 2000s significantly reduced the incidence of CMV infections among HIV patients.

The first FDA-approved (2013) systemically administered ASO is mipomersen (Kastle/Ionis), which is a second generation 2′-O-methoxyethyl containing ASO that is formulated as a sodium adduct for clinical administration. It is a cholesterol-reducing ASO approved as an adjunct to diet and other lipid-lowing drugs for the treatment of homozygous familial hypercholesterolemia, a rare inherited disorder that result in high low density lipoprotein (LDL) cholesterol concentrations and prematurely develop coronary disease (NCT00770146; NCT00794664). Mipomersen targets apolipoprotein B 100 mRNA to block hepatic production of apolipoprotein B-100. This mechanism reduces the levels of very low-density lipoprotein (vLDL) and LDL. Despite mipomersen was effective considering the lipid profile, the therapy was associated with increased risk of discontinuation as well as increased risk of injection-site reactions, hepatotoxicity and flu-like symptoms (Fogacci et al., 2019). For this reason, both in 2012 and 2013 the European Medicines Agency (EMA) concluded that the benefits of mipomersen did not outweigh its risks in the European context, leading to its refusal for marketing authorization in Europe. Mipomersen was also withdrawn from the market in the US by FDA in 2020 considering the concerns about hepatotoxicity (liver damage and elevated liver enzymes).

Eteplirsen (Sarepta Therapeutics) is an ASO approved by FDA in 2016 for the treatment of Duchenne muscular dystrophy (DMD), a genetic disorder characterized by progressive muscle degeneration (Arechavala-Gomeza et al., 2012). Eteplirsen is a splice-switching ASOs designed to hybridize to exon 51 of DMD gene facilitating exon skipping during splicing (Takeda, Clemens, & Hoffman, 2021). This exon skipping restores the reading frame of the dystrophin gene, allowing the production of a shorter but functional dystrophin protein, which helps stabilize muscle fibers and slow disease progression (Lim, Maruyama, & Yokota, 2017). It is estimated to be applicable to about 13–14 % of DMD patients with specific mutations. The success of eteplirsen established a new era for splice-switching ASOs-based therapy in DMD, leading to the approval of three more ASOs: golodirsen (Sarepta Therapeutics), viltolarsen (Nippon Shinyaku), and casimersen (Sarepta Therapeutics). Golodirsen and viltolarsen are ASOs designed to skip exon 53, while casimersen skips exon 45, thereby restoring dystrophin expression in patients with specific mutations. These three drugs received accelerated approval from the FDA in 2019, 2020, and 2021 respectively; therefore, clinical studies are still ongoing to evaluate the risk-benefit profile and long-term effects of this RNA-based therapies in DMD patients (NCT02500381; NCT06606340; NCT04687020; NCT04768062) (Aartsma-Rus & Corey, 2020).

In 2016, the FDA and EMA approved the 18 bp ASO nusinersen (Ionis/Biogen). Nusinersen is the first drug approved by both agencies for the treatment of SMA, an autosomal recessive neuromuscular disease characterized by the degeneration of motor neurons. The disorder is caused by a deletion or loss-of-function mutation in the survival motor neuron 1 (SMN1) gene. Humans also possess a nearly identical gene, SMN2, which differs from SMN1 by a single nucleotide variant in intron 7, encoding a less stable protein unable to fully compensate for the loss of the SMN1 product. Nusinersen is a splice-altering ASO, which binds to the intronic splice silencing site-1 (ISS-1) on the SMN2 pre-mRNA. This binding disrupts the interaction between the ISS-1 and HNRNPLs, which otherwise block the inclusion of exon 7 in the mature mRNA. The inclusion of exon 7 in the mature mRNA allows for the production of full-length SMN protein, which is crucial for proper motor neuron function and is deficient in SMA (Hua et al., 2010; Li, 2020). Nusinersen can improve or stabilize motor function in SMA patients (Neil & Bisaccia, 2019). In particular, clinical trials (ENDEAR, NCT02193074 and CHERISH, NCT02292537) demonstrated that nusinersen significantly improves motor function and survival in infants and children suffering from SMA, especially in early-onset types (Finkel et al., 2017; Mercuri et al., 2018). Nusinersen is administered via intrathecal injection directly into the cerebrospinal fluid to bypass the BBB and reach motor neurons in the CNS. This delivery route is necessary considering the large molecular size and poor CNS penetration of ASOs when administered systemically. The common adverse drug reactions (ADRs) induced by nusinersen include constipation and respiratory infections. Despite its clinical success, nusinersen shows clinical limitations, including the requirement for repeated intrathecal administrations, which may decrease patient compliance and increase the risk of complications (Peng et al., 2025). Additionally, the efficacy of nusinersen varies depending on the age of onset and the type of SMA, highlighting the need for complementary therapeutic approaches (Lemska et al., 2024). The limited availability in some regions as well as the high cost of the treatment are also critical issues (Jalali et al., 2020).

Inotersen (Ionis) is an ASO approved by the FDA and EMA in 2018 for the treatment of hereditary transthyretin amyloidosis (hATTR), a rare genetic disorder caused by single-point mutations in the transthyretin (TTR) gene leading to the production of abnormal protein variants, which pathologically aggregate in amyloid fibrils and accumulate in the heart, peripheral nerves and other organs (Saraiva et al., 1984). Inotersen acts by binding specifically to TTR mRNA, thereby decreasing the concentrations of both mutant and wild-type variants of circulating TTR. Specifically, inotersen exerts its effect through a RNAse H1-dependent mechanism (Gales, 2019). Inotersen effectively reduced serum TTR levels and improved symptoms of polyneuropathy in hATTR patients enrolled in clinical trials (NEURO-TTR, NCT04136184), demonstrating significant improvements of neuropathy progression (Gales, 2019). However, the treatment can induce serious adverse events, notably thrombocytopenia and glomerulonephritis (Benson et al., 2018), which require rigorous hematologic and renal monitoring to mitigate risks. Additionally, cases of liver enzyme elevations warrant

continued long-term safety evaluation (Severi et al., 2023). Moreover, compared to newer ASOs with improved safety profiles and GalNAc conjugation, the therapeutic window of inotersen appears narrower (Conceicao et al., 2024), limiting its widespread clinical use.

Similarly, eplontersen (Ionis-AstraZeneca) is a new ASO developed for targeting TTR mRNA, which received the approval by FDA in 2024. In 2025, eplontersen has also been approved in the EU for the treatment of hATTR in adults with stage 1 or stage 2 polyneuropathy. Results from NEURO-TTRansform Phase III study (NCT04136184) show that eplontersen induced a sustained improvement of neuropathy impairment and quality of life versus placebo. Eplontersen is an advanced GalNAc-conjugated ASO able to target both mutant and wild-type TTR mRNA, decreasing TTR protein production and subsequent amyloid deposition. Compared to its predecessor inotersen, eplontersen shows approximately 20–30 times greater potency, enabling lower and less frequent dosing, which is associated with an improved safety and tolerability profile. Being a GalNAc-conjugated ASO, the use of eplontersen minimizes systemic exposure and reduces adverse events such as thrombocytopenia and renal toxicity, commonly observed with earlier ASOs. Common adverse reactions reported include vitamin A deficiency and mild gastrointestinal symptoms, necessitating supplementation during treatment. Currently, eplontersen is also under further clinical investigations for the treatment of hATTR- associated cardiomyopathy (NCT04136171) and polyneuropathies (NCT05071300). Overall, eplontersen is a significant therapeutic advancement in hATTR treatment, combining potent efficacy with an improved safety profile that facilitates patient adherence.

In 2019 EMA approved volanesorsen (Ionis Pharmaceuticals), a second-generation ASO, as an adjunct to diet in adults with familial chylomicronemia syndrome (FCS) at high risk for pancreatitis, which show inadequate pharmacological responsiveness to diet and triglyceride lowering therapy. Volanesorsen specifically targets the mRNA of apolipoprotein C-III (apoC-III), a key regulator of plasma triglyceride metabolism, thereby inhibiting its synthesis and leading to a reduction of triglyceride levels. However, despite its approval for commercial use in the EU, the FDA declined to grant approval due to safety concerns and an unfavorable risk-benefit profile (NCT02211209) (Gallo et al., 2020; Ghantous et al., 2020; Paik & Duggan, 2019; Witztum et al., 2019). The primary concerns raised by the FDA were related to the risk of thrombocytopenia. Due to this risk, patients treated with volanesorsen require regular monitoring of their platelet counts. In this regard, the FDA recently (2024) approved olezarsen (Ionis Pharmaceuticals), which is a novel ASO conjugated with GalNAc3 developed for the treatment of FCS. Olezarsen, like volanesorsen, acts by targeting and degrading the mRNA of apoC-III (Karwatowska-Prokopczuk et al., 2024) but its administration is associated with a lower risk of thrombocytopenia compared volanesorsen. This is ascribed to the fact that the GalNAc3 conjugation leads to a more efficient and targeted delivery to the liver, which translates to a lower effective dose and less frequent administration (e.g., once monthly for olezarsen vs. weekly initially, then bi-weekly for volanesorsen). In a phase III, double-blind, placebo-controlled trial (the BALANCE trial; NCT04568434), olezarsen administered every 4 weeks significantly reduced fasting triglyceride levels as well as the frequency of acute pancreatitis episodes in patients with genetically confirmed FCS compared to the placebo group (Stroes et al., 2024). Studies have explored the transition of patients with FCS from volanesorsen to olezarsen, with olezarsen generally being well-tolerated after a washout period (Gaudet et al., 2024). This suggests that olezarsen may offer a favorable alternative for patients. Olezarsen thus represents a significant advance in the treatment of severe hypertriglyceridemia, especially for patients with FCS at high risk for recurrent pancreatitis and for whom available therapies have limited effectiveness. However, further studies are needed to confirm its impact on long-term cardiovascular outcomes.

The superoxide dismutase 1 (SOD1)-targeting ASO tofersen (Ionis/Biogen) received approval from FDA and EMA in 2023 for the treatment of amyotrophic lateral sclerosis (ALS) associated with mutations in the SOD1 gene (Everett & Bucelli, 2024). In the VALOR Phase III clinical trial (NCT02623699), tofersen administration significantly reduced SOD1 protein levels in the CSF and slowed disease progression inducing minimal adverse effects. Tofersen binds to SOD1 mRNA and forms a RNA-DNA hybrid, which is a substrate for RNase H, an enzyme that degrades RNA (Saini & Chawla, 2024). In particular, tofersen is injected intrathecally into the cerebrospinal fluid (CSF) in order to specifically reduce the amount of SOD1 protein produced by affected motor neurons. However, the treatment consists of intrathecal administration every four weeks, which can be challenging for patients due to the invasive procedure and associated risks (Lovett et al., 2025). While tofersen offers the first precision therapy targeting a genetic cause of ALS, further long-term evaluation is needed to assess broader clinical use, and impact on overall survival (Simonini et al., 2025).

Imetelstat (Geron Corporation) is a first-in-class 13-mer ASO telomerase inhibitor recently approved (2024) by FDA and EMA (2025). It was approved for treating adult patients with lower-risk myelodysplastic syndromes (LR-MDS) who have transfusion-dependent anemia requiring at least four red blood cell units over 8 weeks, and who are refractory to, have lost response to, or are ineligible for erythropoiesis-stimulating agents (ESA). It is administered intravenously typically over 2 h every 4 weeks. Imetelstat inhibits telomerase activity by a competitive binding to its RNA template region. This mechanism targets the elevated telomerase activity, which is found in most cancers and neoplastic progenitor cells helps maintain cellular immortality (Lennox et al., 2024). Clinical trials, such as the pivotal IMerge trial (NCT02598661), reported that imetelstat significantly ameliorated red blood cell transfusion independence ($\geq$8 weeks and $\geq$ 24 weeks) compared to placebo, with a controllable safety profile mainly involving short-lived neutropenia and thrombocytopenia (Platzbecker et al., 2024). The intravenous administration every 4 weeks may reduce however patient compliance compared to therapies involving subcutaneous delivery. Further studies are ongoing to assess the efficacy of imetelstat in other hematologic conditions and long-term outcomes (Lennox et al., 2024).

Donidalorsen (Ionis Pharmaceuticals) is a RNA-targeted ASO recently approved (2025) by the FDA for the prophylactic treatment of hereditary angioedema (HAE) in adults and pediatric patients aged 12 years and older. HAE is a rare and potentially life-threatening genetic disease characterized by frequent, unpredictable attacks of severe swelling in several parts of the body (hands, feet, face, genitals, stomach, and throat). Swelling of the throat can be dangerous because it can produce airway obstruction. Donidalorsen is a Ligand-Conjugated Antisense (LICA) oligonucleotide, which targets the mRNA that encodes for prekallikrein (PKK). PKK is a key protein, which contribute to activate inflammatory mediators, including bradykinin, which lead to HAE attacks. By binding to the PKK mRNA, the drug successfully decreases the liver production of PKK. This reduction in PKK levels decreases the levels of kallikrein and subsequently bradykinin in the bloodstream, thereby blocking the pathway that causes the swelling attacks in HAE patients (Farkas & Balla, 2024). The approval of donidalorsen mainly depended on its successful evaluation in Phase III clinical trials, notably the OASIS-HAE study (NCT05139810) and its open-label extension, OASISplus (Riedl et al., 2024). These key clinical trials demonstrated significant and sustained reductions in HAE attack rates up to 96 % mean reduction maintained for up to three years (Riedl et al., 2025). The drug is injected via subcutaneous administration, typically every four or eight weeks. Further investigations however should aim to optimize the dosing schedule to maximize patient benefit, especially considering that the 4-week regimen demonstrated superior efficacy in reducing attack frequency compared to the 8-week interval (Riedl et al., 2024).

4.2. Investigational ASO-based drugs

A significant milestone demonstrating the unlimited potential of RNA-based therapeutics is the development of milasen, which

represents a new era of personalized medicine. Milasen was developed by Boston Children's Hospital for Mila Makovec, a young patient with a rare form of Batten disease, caused by a specific mutation in the CNL7 gene, leading to the mis-splicing of exon 6 (Kim et al., 2019). This disorder is characterized by early-onset seizures, vision loss, and developmental decline, with few patients surviving beyond their teenage years (Wilton-Clark, Yan, & Yokota, 2024). Milasen was designed, based on nusinersen structure, to bind faulty RNA transcript in cells of Mila, avoiding the premature halting of the RNA splicing process (Kim et al., 2019). In particular, a retrotransposon insertion in CLN7 gene (also known as MFSD8) of Mila leads to the production of a non-functional protein. Milasen acts correcting this genetic error by enabling the splicing of the correct coding regions. The treatment reduced the frequency and duration of seizures by half, with no serious adverse effects reported (Kim et al., 2019). However, it did not block overall neurodegeneration, and Mila passed away three years after starting therapy. Milasen was administered as an "N-of-1" investigational therapy, with FDA permission for single-patient use, establishing a precedent for future individualized ASO treatments (The cost of getting personal, 2019). Milasen demonstrated the feasibility of rapid, patient-specific drug development for ultra-rare genetic diseases, influencing the creation of FDA guidelines for N-of-1 antisense therapies and inspiring similar personalized approaches for other conditions. The development of milasen represents a landmark example of precision medicine.

Ulefnersen (Ionis Pharmaceuticals; Licensed to Otsuka Pharmaceutical) is an investigational ASO currently being evaluated in a Phase III clinical trial (FUSION; NCT04768972) for ALS but it is specifically designed to reduce the expression of fused in sarcoma (FUS) RNA binding protein. The P525L FUS variant leads to the development of a rare and rapidly progressing juvenile form of ALS defined FUS-ALS (Korobeynikov et al., 2022). The non-allele-specific silencing of The P525L FUS variant expression by ulefnersen represents a novel and specific mechanism to decrease the loss of motor neurons and the disease progression in FUS-ALS patients. The drug is administered via intrathecal injection every 12 weeks, allowing targeted delivery. Since 2019, ulefnersen has been administered to over 25 patients worldwide under compassionate use or expanded access programs, demonstrating promising effectiveness in decreasing disease progression and improving symptoms. In this regard, ulefnersen has received orphan drug designation (2023) from both the FDA and EMA, which provided regulatory incentives to accelerate its development for this rare disease. Ulefnersen may thus represent a precision therapeutic breakthrough for a rare, aggressive familial ALS subtype. While translational issues and clinical outcome variability persist, further studies are necessary to fully establish its therapeutic potential and safety profile. For instance, the long-term effects of FUS protein reduction need to be assessed given the role of FUS in RNA processing and DNA repair (Zhou et al., 2014).

Clinical studies utilizing ASOs have been conducted for Huntington's disease (HD), an inherited autosomal dominant disorder caused by a mutation in the Huntingtin (HTT) gene, which may increase the repeat count and result in a defective protein, leading to progressive neurodegeneration (Walker, 2007). Tominersen (Roche and Ionis Pharmaceuticals) is an ASO designed to bind to the mRNA that carries the genetic instructions for making the HTT protein. It targets both the normal (wild-type) and the mutated (mHTT) forms of the mRNA. By binding to the HTT mRNA, tominersen triggers a RNase H-mediated degradation that prevents the mRNA from being translated into protein in the brain. The goal of the pharmacological treatment is to slow or block the neurodegeneration characteristic of HD by lowering the concentration of the toxic mHTT. (McColgan et al., 2023). Tominersen is administered via intrathecal administration as other ASOs, which are large and charged molecules not capable to readily cross the BBB. Tominersen was evaluated in a Phase III study called GENERATION HD (NCT03761849), which however was blocked because safety risks outweighed any potential benefits. In this regard, a "post hoc" analysis revealed that individuals with less severe symptoms who started the trial at a younger age may show responsiveness to tominersen. For this reason, the pharmaceutical company in 2023 started a new randomized, double-blind, placebo-controlled, dose-finding Phase II clinical trial, which is ongoing, targeting a more defined patient population with prodromal and early manifest HD (GENERATION HD2; NCT05686551). Importantly, the failure of tominersen underscored the risks of non-allele-specific HTT suppression. Indeed, wild-type HTT (wtHTT) plays essential roles in neuronal development, survival, and function (Gauthier et al., 2004; Rigamonti et al., 2000). The indiscriminate reduction of both wtHTT and mHTT induced by tominersen may have contributed to adverse neurological effects and limited clinical effectiveness. This stands in stark contrast to the first-in-class ASO WVE- 003 (Wave Life Sciences), which is capable of selectively silence mHTT by targeting a single nucleotide polymorphism specific for the mutant allele (Iwamoto et al., 2024). WVE- 003 has recently completed a Phase I/II clinical trial involving Huntington patients with specific HTT variants. Results from the SELECT-HD (NCT05032196) study demonstrated significant and long-lasting allele-selective silencing, with a 46 % mean reduction exclusively in mHTT with a generally safe and well-tolerated profile, preserving wtHTT expression. Overall, the contrast between non-selective pan-HTT suppression and allele-specific targeting suggests the importance of molecular selectivity in developing safe and effective RNA-based drugs for HD and other similar diseases. The insights obtained from the clinical challenges related to tominersen have thus accelerated a strategic shift toward allele-specific approaches, which aim to ameliorate therapeutic efficacy and minimizing potential neurotoxicity.

Zorevunersen (STK-001), is the first disease-modifying ASO developed for the treatment of Dravet syndrome, a developmental and epileptic encephalopathy caused by haploinsufficiency of the sodium channel gene SCN1A. Specifically, zorevunersen is designed to upregulate NaV1.1 protein expression by preventing the expression of exon 20 N in the non-mutant (wild-type) copy of the SCN1A gene (Yuan et al., 2024). This mechanism should restores physiological NaV1.1 levels and ultimately reduces both occurrence of seizures and significant non-seizure comorbidities (Han et al., 2020). The clinical effectiveness of zorevunersen is under investigation in two clinical studies (NCT04740476; NCT06872125). Clinical data importantly report persistent reductions in convulsive seizure frequency, improvements in cognition and behavior, and sustained upregulation of NaV1.1 protein (Samanta et al., 2025). Zorevunersen has showed the potential for working as disease modifying drug and thus it has been granted orphan drug designation by the FDA and the EMA.

ProQR Therapeutics and Laboratoires Théa have developed two ASOs candidates, sepofarsen and ultevursen for the treatment of genetic retinal diseases. In particular, sepofarsen is under investigation for the treatment of Leber congenital amaurosis type 10 (LCA10), which is caused by a specific mutation in the CEP290 gene (c.2991 + 1655 A > G, also referred to as p.Cys998X). LCA10 is a severe inherited retinal disease that leads to childhood blindness (Cideciyan et al., 2021). Sepofarsen (also known as QR-110) is a RNA ASO that targets the deep intronic c.2991 + 1655 A > G variant in intron 26 of the CEP290 gene. This mutation creates a "cryptic" or "pseudoexon" splicing site, leading to the insertion of an extra exon into the CEP290 mRNA. This aberrant splicing results in a premature stop codon, leading to either nonsense-mediated mRNA decay or the production of a truncated, non-functional CEP290 protein (Russell et al., 2022). The CEP290 protein is vital for the formation and stability of the connecting cilium within retinal photoreceptor cells and thus for a proper vision. Sepofarsen increases the amount of wild-type CEP290 mRNA, leading to increased production of functional CEP290 protein in retinal photoreceptors by correcting the aberrant splicing. Results from a Phase Ib/II trial (NCT03140969) report that sepofarsen was generally well tolerated, with most adverse events being mild and dose-dependent (Russell et al., 2022). Sepofarsen induced a significant improvement in visual acuity and retinal sensitivity, with almost half of the patients achieving a

clinically meaningful gain. Importantly, this improvement was maintained over a 12-month follow-up period. Sepofarsen has received orphan drug designation in both the US and EU, as well as fast-track and rare pediatric disease designations from the FDA. Currently, sepofarsen is under investigation in a pivotal Phase II/III trial (ILLUMINATE; NCT03913143) randomized, double-blind, sham-controlled study involving both adults and children with LCA10 due to the CEP290 c.2991 + 1655 A > G mutation.

Ultevursen is currently under investigation for Usher syndrome type 2, or retinitis pigmentosa, characterized by a progressive loss of visual function (Dulla et al., 2021). Ultevursen is a splice-switching ASO designed to target exon 13 of the USH2A gene, which is responsible for blocking the production of usherin, an essential protein for retinal function. Ultevursen aims to restore the production of a functional usherin protein, by inducing exon 13 skipping. This is hypothesized to improve the functionality of retinal photoreceptor cells. Ultevursen is in Phase II clinical development (NCT05158296).

Several studies assessing RNA-based drugs have interestingly reported promising results also for difficult-to-treat neurodegenerative diseases including Alzheimer's disease (AD). $MAPT_{Rx}$ (Biogen and Ionis Pharmaceutics), is an ASO designed to reduce concentrations of MAPT mRNA, which encodes for tau protein. Accumulating evidence suggests that lowering the amount of tau protein may be an effective strategy for treating AD (Mummery et al., 2023). More specifically, $MAPT_{Rx}$ targets a specific 18-nucleotide stretch within intron 9 of the MAPT pre-mRNA and promotes its degradation by recruiting the RNAse H1. This leads to a reduction in the amount of tau protein synthesized (Fig. 4). By decreasing tau levels, MAPTRx can reduce tau pathology and potentially modify disease progression in AD and other tauopathies. Encouraging results from a Phase Ib clinical trial (NCT03186989) have underscored the therapeutic potential of MAPTRx in treating AD (Mummery et al., 2023). In particular, there were no serious adverse events among patients intrathecally administered with MAPTRx. Importantly, MAPTRx induced a dose-dependent decrease (more than 50 %) in the CSF total-tau concentration from baseline at 24 weeks after the last dose. $MAPT_{Rx}$ is also currently under investigation in a Phase II Clinical trial (NCT05399888).

Pelacarsen (Ionis Pharmaceuticals and Novartis) is a promising third-generation ASO specifically designed to lower elevated levels of apolipoprotein(a) [apo(a)] in individuals at heightened risk for cardiovascular diseases (CVDs). Pelacarsen is designed utilizing LICA technology platform of Ionis Pharmaceuticals. The LICA technology, which includes GalNAc3 conjugation, enables targeted delivery of pelacarsen to hepatocytes, improves stability and prolongs its action with minimal off-target effects. Moreover, key chemical modifications including locked nucleic acids (LNAs) and 2′-O-methoxyethyl groups increase the half-life of pelacarsen by protecting the drug from nuclease degradation. The specific target of pelacarsen is the mRNA coding for apo(a), which is mainly produced in the liver. Pelacarsen binds specifically to its complementary sequence on the apo(a) mRNA in liver cells. This binding forms a DNA-RNA hybrid that is recognized and cleaved by endogenous RNase H enzymes. By degrading the apo(a) mRNA, pelacarsen prevents its translation into the apo(a) protein. Previous studies have confirmed the efficacy of pelacarsen in reducing lipoprotein(a) levels in 90 % of patients, with no significant side effects or toxicity reported (NCT03070782) (Tsimikas et al., 2020). It is currently evaluated in two Phase III studies (NCT05900141; NCT06267560) for patients with hyperlipoproteinemia and established atherosclerotic CVDs. But most importantly, the efficacy and safety of pelacarsen is being evaluated in the Lp(a) HORIZON study (NCT 04023552), which is a large phase III, randomized, double-blind, placebo-controlled multinational trial involving 8323 patients with established CVD and elevated Lp(a) levels

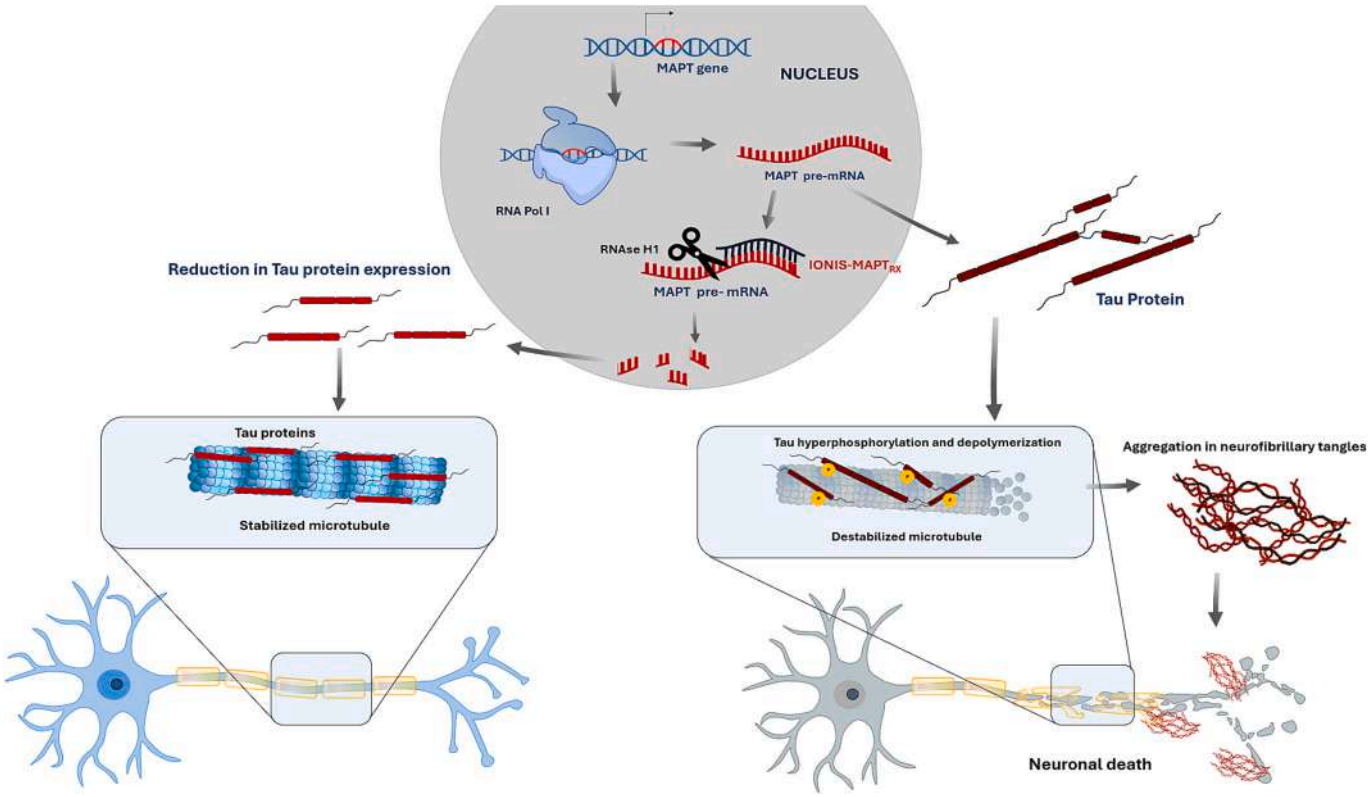

Fig. 4. Mechanism of action of $MAPT_{RX}$.
$MAPT_{RX}$ is an ASO designed to reduce tau protein levels in the brain. Tau protein typically serves to stabilize microtubules in neurons; however, in AD and other tauopathies, tau becomes hyperphosphorylated, misfolds, and aggregates, forming neurofibrillary tangles that contribute to neuronal dysfunction and cell death. Upon administration, $MAPT_{RX,}$ enters neurons and binds complementarily to the mRNA transcript of the MAPT gene. The binding of $MAPT_{RX}$ to the target mRNA recruits RNase H, which cleaves and degrades MAPT mRNA, thereby reducing Tau protein production.

of ≥70 mg/dL (Cho et al., 2025). Although pelacarsen achieves robust reductions in apo(a) levels, its impact on major cardiovascular clinical outcomes such as death, myocardial infarction, and stroke remains to be definitively demonstrated in the ongoing studies. Furthermore, considerations regarding cost-effectiveness and patient access are important given the chronic management needs of CVDs. Nonetheless, pelacarsen may be a crucial first-in-class ASO considering that currently there are no approved pharmacological treatments to successfully lower apo(a).

Vupanorsen (Pfizer and Ionis Pharmaceuticals) is a second-generation GalNAc3-modified ASO designed to target and inhibit the hepatic mRNA coding for angiopoietin-like 3 (ANGPTL3), a key regulator of triglyceride and cholesterol metabolism. ANGPTL3 basically inhibits enzymes like lipoprotein lipase (LPL) that degrades triglycerides. By reducing ANGPTL3, vupanorsen would disinhibit LPL, leading to lower triglyceride levels. Despite meeting its primary and key secondary endpoints in the Phase IIb TRANSLATE-TIMI 70 study (NCT04516291), Pfizer decided to discontinue the program in 2022 for several reasons. Whereas vupanorsen effectively lowered triglycerides and ANGPTL3, the magnitude of reduction in non-HDL-C and LDL-C was considered less than hoped for and likely insufficient to support further large-scale Phase III studies for cardiovascular risk reduction. In this respect, larger reductions in atherogenic lipoproteins are generally expected for a drug to significantly impact cardiovascular outcomes. The development of vupanorsen was discontinued also in consequence of dose-dependent elevations in liver enzymes (ALT, AST) and increases in hepatic fat fraction (Bergmark et al., 2022; Gaudet et al., 2020). These safety concerns exemplify a broader challenge of ASO-mediated hepatotoxicity, which arises from both hybridization-dependent mechanisms, including off-target RNase H-mediated cleavage of unintended RNAs, and hybridization-independent pathways, such as chemical and immune-mediated toxicities within the liver (Zimerman et al., 2024). This failure emphasizes valuable insights for the development of next-generation ASOs, including the necessity to refine chemical modifications, optimize dosing schedules, and improve targeting strategies to decrease hepatic toxicity (Oostveen, Hovingh, & Stroes, 2023). Current innovations focus on enhancing ASO pharmacokinetics and minimizing off-target effects including liver-related side effects while maintaining therapeutic effectiveness (Collotta et al., 2023). The clinical experience with vupanorsen underscores the critical need for early, rigorous assessment of hepatotoxicity in developing ASO-based drugs for metabolic disorders to achieve an optimal balance between efficacy and safety.

Interestingly IONIS-AGT-LRx (also known as evazarsen sodium; Ionis Pharmaceuticals) is an ASO under evaluation as a monotherapy (NCT04714320; NCT04083222) for the treatment of hypertension (especially treatment-resistant hypertension) and heart failure. IONIS-AGT-LRx is a hepatocyte-directed GalNAc3-modified ASO that binds to the angiotensinogen (AGT) mRNA in a sequence-specific manner. This binding leads to the RNase H1-dependent degradation of the AGT mRNA and to the consequent reduction of circulating AGT levels (Benigni, Cassis, & Remuzzi, 2010; Morgan et al., 2021). The development of IONIS-AGT-LRx may represent a novel approach to renin-angiotensin-aldosterone system inhibition, potentially effective for patients who do not achieve adequate blood pressure control with available pharmacotherapies. However, larger studies are needed to assess the clinical efficacy of IONIS-AGT-LRx.

Diabetes is one of the most widespread metabolic disorders worldwide, characterized by high blood glucose levels due to insulin deficiency (Type 1) and insulin resistance (Type 2) (Reaven, 1988). IONIS-$GCGR_{RX}$ (Ionis Pharmaceuticals) is a 2'-*O*-methoxyethyl ASO designed to selectively target human glucagon receptor pre-miRNA, thereby reducing its hepatic expression. Glucagon counteracts the effects of insulin and stimulates the hepatic production of glucose. In type 2 diabetes, elevated glucagon levels contribute significantly to hyperglycemia. IONIS-GCGRRx, by reducing the number of glucagon receptors, decreases the impact of glucagon on the liver thereby lowering hepatic glucose output and ultimately reducing blood glucose levels. IONIS-$GCGR_{RX}$ is currently being evaluated in three different Phase II clinical studies (NCT02583919; NCT02824003; NCT01885260); preliminary findings in this regard indicate that the candidate ASO successfully inhibit glucagon receptor expression, showing improvements in glycemic control even at low doses. Although no significant side effects, such as hypoglycemia, have been reported (Morgan et al., 2019), the safety profile of IONIS-GCGRRx require further evaluation to confirm clinical viability and balance potential risks.

The most common hepatic manifestation of metabolic syndrome is nonalcoholic fatty liver disease (NAFLD) that can range in severity from simple fatty liver (steatosis) to metabolic dysfunction-associated steatohepatitis (MASH), and, eventually, liver cirrhosis (Younossi et al., 2016). Nevertheless, the availability of disease-modifying medications is currently limited, and treatment options for MASH, are mainly focused on lifestyle adjustments (Younossi et al., 2019). Two ASO-based drugs are currently being evaluated in clinical trials for the treatment of MASH: ION224 in phase II (NCT04932512), AZD2693 in phase II (NCT05809934). ION224 (Ionis Pharmaceuticals) is a LICA developed to inhibit the production of diacylglycerol acyltransferase 2 (DGAT2) enzyme. DGAT2 plays a crucial role in the final step of hepatic triglyceride synthesis. ION224 decreases the overproduction of triglycerides, which contributes to excess liver fat, inflammation, and damage observed in MASH by inhibiting DGAT2. A Phase II clinical trial (NCT04932512) assessing ION224 demonstrated positive outcomes, including an improvement in the NAFLD Activity Score (NAS), while showing no worsening of fibrosis. ION224 showed a favorable safety profile, positioning it as a promising therapeutic candidate for MASH (Loomba et al., 2025). However, confirmatory phase III trials are needed to establish long-term efficacy and safety. AZD2693 (AstraZeneca) is an ASO that targets the PNPLA3 gene (Patatin-like phospholipase domain containing 3), which encodes a protein that plays a crucial role in the progression of fatty liver disease. A common genetic variant, PNPLA3 rs738409 148 M, is recognized as the largest single genetic risk factor for the progression of fatty liver disease to MASH, fibrosis, cirrhosis and liver-related mortality. AZD2693 decreases the production of the PNPLA3 protein by reducing the mRNA expression of PNPLA3 gene in the liver (Armisen et al., 2025). This mechanism mitigates liver fat accumulation, inflammation and fibrosis. AZD2693 is currently under clinical evaluation in a Phase IIb randomized, double-blind, placebo-controlled trial (NCT05809934) in adults aged 18–75 years with non-cirrhotic Nonalcoholic steatohepatitis (NASH) and fibrosis (F2 or F3) who carry the PNPLA3 rs738409 148 M risk allele. The development of AZD2693 represents a precision medicine approach because it is specifically designed for individuals carrying the PNPLA3 rs738409 148 M risk allele.

Recently, the FDA has granted the Fast Track designation (2024) to bepirovirsen (GlaxoSmithKlin) in order to accelerate its development for the treatment of hepatitis B, which is a chronic disease caused by the hepatitis B virus (HBV). Bepirovirsen is an ASO designed to recognize and destroy the genetic components (RNA) of the hepatitis B virus that are crucial for its replication and for producing viral proteins, including the hepatitis B surface antigen (HBsAg). The ASO is chemically modified with 2′-O-methoxyethyl groups for stability and enhanced potency. Bepirovirsen effectively inhibits the replication of viral DNA in the body and suppresses the level of HBsAg in the blood by reducing the levels of HBV RNA (Yuen et al., 2021). Additionally, bepirovirsen holds an immunomodulatory property by stimulating immune responses, potentially via toll-like receptor 8 (TLR8). Despite its promising antiviral and immunostimulatory effects, the clinical development of bepirovirsen faces several challenges. Other studies indeed report that bepirovirsen achieves sustained HBsAg and HBV DNA loss in only about 9–10 % of patients in phase IIb trials, highlighting modest efficacy (Yuen et al., 2022a). Transient liver enzyme elevations as well as injection site reactions are common, although generally mild, impacting tolerability

(Yuen et al., 2022a). Bepirovirsen is however currently under investigation in two Phase III studies (NCT05630807, NCT05630820).

Miravirsen (Santaris Pharma and Roche) is a significant drug in the history of RNA-based drugs, because it was the first microRNA-targeted drug to enter human clinical trials. Miravirsen was developed for the treatment of chronic hepatitis C virus (HCV) infection. Miravirsen is an ASO targeting the microRNA-122 (miR-122), which is a highly expressed liver-specific miRNA essential for the HCV life cycle. Miravirsen indirectly suppresses HCV replication and reduces viral load by inhibiting miR-122. While Miravirsen showed promising antiviral activity and was generally well-tolerated, its development for HCV was ultimately superseded. This was due to the rapid and revolutionary development of direct-acting antiviral therapies for HCV, which achieved very high cure rates (often >95 %) with shorter treatment durations and fewer side effects. Although miravirsen did not reach commercial approval, its development was crucial for validating the concept of microRNA-targeted therapeutics. Its clinical development demonstrated that targeting host microRNAs could be an effective antiviral strategy. This pioneering work has promoted the development of other miRNA-based drugs and ASO therapies for various diseases beyond viral infections.

4.3. Approved mRNA vaccines

The SARS-CoV-2 pandemic led to an unexpected exploit in the field of RNA therapeutics. In this respect, mRNA vaccines have undergone clinical trials for oncologic therapies for almost 10 years (Fiedler et al., 2016), and for infectious disease for over 3 years (Feldman et al., 2019). The findings obtained in many years of preclinical and clinical investigation regarding safety, tolerability, and efficacy have played a pivotal role in accelerating the development of COVID-19 vaccines. The first mRNA vaccine BNT162b2 (Pfizer-BioNtech) settled a milestone in the field of vaccine development. Following this significant advance, mRNA-1273 (Moderna), a second SARS-Cov-2 mRNA vaccine using LNP delivery technology, was subsequently developed further demonstrating the immense potential of mRNA technology. Both BNT162b2 and mRNA-1273 demonstrated very high efficacy (around 95 % in initial trials) against symptomatic COVID-19 and severe disease, hospitalization and death (Polack et al., 2020; Thompson et al., 2021). These vaccines received emergency use authorizations (2020) and subsequently full regulatory approvals from major health authorities worldwide. Unlike traditional vaccines that introduce a weakened or inactivated virus or viral components, mRNA vaccines deliver the mRNA carrying the genetic blueprint for a harmless but distinctive protein found on the surface of the SARS-CoV-2 virus, the full-length S2—P spike viral protein, responsible for receptor binding and subsequent fusion with the host cell membrane (Xu et al., 2022). The mRNA is protected from degradation by encapsulation in LNPs. Once administered, the LNPs fuse with the muscle cells and release the mRNA, which is translated in SARS-CoV-2 spike protein. These spike proteins are displayed on the surface of the cells and activate the immune response. This involves production of antibodies for spike protein neutralizing the virus if a real infection occurs and T-cell activation to recognize and destroy cells infected with the virus. The mRNA is naturally broken down and eliminated within a short period (Liu et al., 2020; Sadarangani, Marchant, & Kollmann, 2021). The mRNA platform allows for rapid vaccine development, which was crucial during the pandemic. Once the genetic sequence of a target antigen (like the SARS-CoV-2 spike protein) of pathogens is known, the mRNA can be designed and synthesized quickly in a laboratory setting. Compared to traditional vaccine production methods, manufacturing can also be scaled up quickly enough. Regarding the safety profile, mRNA vaccines have generally a favorable safety profile inducing mild to moderate and temporary side effects (e.g., pain at injection site, fatigue, headache, fever). While rare severe ADRs like myocarditis and pericarditis have been observed (mostly in young males), the overall benefits of vaccination undoubtedly outweigh the risks. Another significant advantage of mRNA technology is its adaptability considering the arising of SARS-CoV-2 variants. Indeed, the mRNA sequence in the vaccine can be rapidly updated to match the new spike protein variants. This has led to the development of updated mRNA vaccines to provide better protection against currently circulating strains. Notwithstanding their successes, critical differences and challenges persist regarding BNT162b2 and mRNA-1273. The mRNA platform of Moderna employs a higher mRNA dose per injection (100 μg in original formulation) than Pfizer-BioNTech's 30 μg dose, potentially contributing to marginally more robust immune responses but also to increased reactogenicity and ADRs frequency (Akingbola et al., 2025a). While both vaccines share typical side effects, mRNA-1273 is sometimes associated with higher rates of transient systemic reactions such as fever and fatigue, which may impact patient acceptance. In summary, BNT162b2 and mRNA-1273 remain cornerstone COVID-19 vaccines, yet their evolving formulations, dosing differences, reactogenicity and safety profiles demand continued critical evaluation to optimize their global use.

ARCT-154 (Arcturus Therapeutics in partnership with CSL Seqirus and Meiji Seika Pharma) represents a significant progress in COVID-19 vaccines. The fundamental innovation of ARCT-154 relies on its self-amplifying mRNA platform. Differently from conventional mRNA vaccines (like BNT162b2 and mRNA-1273), the mRNA of ARCT-154 encodes for SARS-CoV-2 spike protein as well as for replicase enzymes, which then create multiple copies of the mRNA vaccine within the cell. In comparison with non-amplifying mRNA vaccines, this self-amplification induces a higher and more sustained production of the spike protein even at low initial dose (Ho et al., 2024). The rationale is to increase the antigen production and induce a more potent, broader, and potentially more long-lasting immune response, including neutralizing antibodies and T-cell responses, with a reduction of the mRNA needed per dose. Studies have revealed that ARCT-154 can induce long-lasting immune responses (antibody levels persisting for at least 12 months post-vaccination) (Oda et al., 2024). Interestingly, ARCT-154 produces extensive immunogenicity against several SARS-CoV-2 variants such as Omicron sub-lineages (BA.4/5 and JN.1) (Ho et al., 2025). Clinical trials (NCT05012943; NCT05037097) suggest that ARCT-154 is generally well-tolerated, having a safety profile similar to other mRNA vaccines. No reports of myocarditis or pericarditis have been associated with the administration of ARCT-154. ARCT-154 has achieved significant regulatory milestones: it was approved in 2023 as the world's first approved self-amplifying mRNA vaccine by Japan's Ministry of Health, Labour and Welfare for primary vaccination and booster doses in adults 18 years and older. The European Commission granted marketing authorization for ARCT-154 in 2025 for individuals 18 years and older. It has also received regulatory approval in Vietnam. These approvals recognize the self-amplifying mRNA platform as a valuable therapeutic tool, which can offer important advantages in terms of dosing, durability, and adaptability to new SARS-CoV-2 variants.

mRNA-1345 (Moderna) is an mRNA vaccine developed for the prevention of lower respiratory tract disease (LRTD) caused by respiratory syncytial virus (RSV). mRNA-1345 received FDA Breakthrough Therapy Designation in 2023 and was approved by FDA in 2024 for adults 60 years and older. The FDA expanded its approval in 2025 to include adults aged 18 to 59 years who are at increased risk for severe RSV-related disease. The European Commission granted marketing authorization for mRNA-1345 in thirty European countries in 2024. The mRNA vaccine has also received authorization in other global markets, including Canada (2024). mRNA-1345 comprises a single mRNA sequence encoding for a stabilized prefusion F glycoprotein of the RSV virus, which is crucial for the virus to enter host cells and is a significant target for neutralizing antibodies. The vaccine is based on the same LNP delivery system used for mRNA-1273. In a Phase III trial (ConquerRSV NCT05127434) performed in older adults, mRNA-1345 exhibited an efficacy of 83.7 % against RSV-LRTD defined by two or more symptoms, and 82.4 % against RSV-LRTD with three or more symptoms (Wilson

et al., 2023). mRNA-1345 was generally well-tolerated. Common reported adverse reactions were mostly mild to moderate (Grade 1 or 2) and included injection site pain, fatigue, headache, myalgia, and arthralgia. Thus, the approval of mRNA-1345 expands the use of mRNA technology beyond COVID-19, providing a valuable tool to reduce RSV-related morbidity and hospitalizations. Although highly promising and potentially transformative for preventing RSV disease in vulnerable populations, mRNA-1345 requires real-world effectiveness monitoring to confirm the persistence of protection and safety. Additionally, comparisons with other RSV vaccine candidates and evaluation in pediatric populations remain important aspects for future investigation (Goswami et al., 2025).

4.4. Investigational mRNA vaccines

mRNA vaccines have recently gained attention and broad support due to their ability to instruct the immune system in identifying and eradicating cancer cells through the administration of mRNA encoding for tumor-specific antigens (Yu et al., 2023). BNT111 (BioNTech), is a tetravalent RNA-lipoplex cancer vaccine designed to target four melanoma tumor-associated antigens: tyrosinase, trans-membrane phosphatase with tensin homology (TPTE), New York-ESO-1, and MAGE-A3. These mRNAs are delivered via an exclusive uridine mRNA-lipoplex (LPX) formulation. BNT111 is designed to be highly immunostimulatory, mimicking a viral infection to alert the immune system. By presenting the blueprint for these four melanoma-associated antigens, BNT111 stimulates the immune system to recognize and attack cancer cells that express one or more of these antigens. The main objective is to create long-lasting immune memory against these common melanoma antigens. The efficacy of BNT111 is currently under investigation in a randomized Phase II clinical trial (NCT04526899) in patients with anti-PD-1-refractory or relapsed unresectable stage III and IV melanoma. In November 2021, BioNTech received FDA Fast Track Designation for BNT111 in patients with stage III or IV melanoma, based on the positive results from the phase 1 trial (NCT02410733). The preliminary results from the Phase II trial are positive and thus sign a significant step for BioNTech's mRNA cancer vaccine technology and its strategy of combining synergistic modalities with established immune checkpoint treatments, particularly for patients with difficult-to-treat, anti-PD-(L)1 refractory or resistant melanoma. However, larger trials are necessary to to further assess the clinical efficacy of BNT111.

BNT113 (BioNTech) is an investigational mRNA-based cancer immunotherapy using the "FixVac" platform developed by BioNTech. It was developed for the treatment of cancers driven by Human Papillomavirus 16 (HPV16). BNT113 is an mRNA-lipoplex cancer vaccine, that encodes for HPV16 oncoproteins E6 and E7, which have potential immunomodulating and antineoplastic activities. These are critical viral proteins that drive the development and progression of HPV16-positive cancers. The mRNA is encapsulated in exclusive RNA-lipoplex formulation of BioNTech, which is designed for intravenous administration. BNT113 is undergoing investigation in a Phase II trial involving patients with unresectable recurrent or metastatic head and neck cancer positive for HPV-16 and expressing the protein PD-L1 (NCT04534205). Results from a previously completed Phase I/II trial (NCT03418480) indicated that BNT113 treatment produces a potent antigen-specific T cell response. Although further investigation is needed, the development of BNT113 may address a significant unmet need in HPV16-positive cancers, particularly in patients showing immunotherapy resistance.

BNT116 (BioNTech) is an mRNA cancer vaccine candidate encoding for six tumor-associated antigens (MAGE-A3, CLDN6, KK-LC-1, PRAME, MAGE-A4, and MAGE-C1) expressed in non-small cell lung cancer (NSCLC). The mRNA vaccine is formulated as an RNA-lipoplex and is administered intravenously. Also in this case, BNT116 "trains" the immune system of the patient, specifically cytotoxic T cells, to recognize

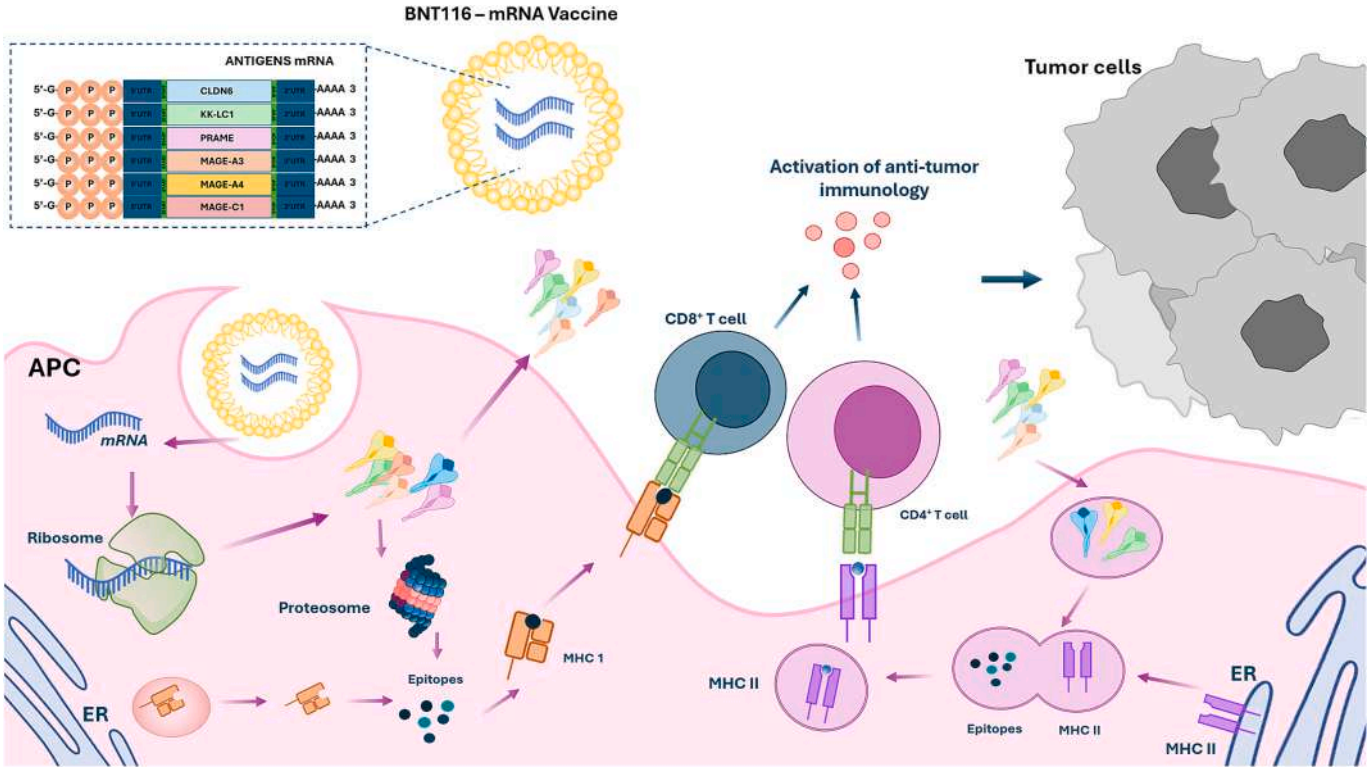

Fig. 5. Mechanism of action of BNT116 mRNA cancer vaccine.
The BNT116 mRNA vaccine encodes six shared tumor-associated antigens (CLDN6, KK-LC-1, PRAME, MAGE-A3, MAGE-A4, and MAGE-C1) frequently found in NSCLC. The mRNA is taken up by APCs, which translate the encoded antigens. These antigens are presented to T cells, initiating tumor-specific immunity by stimulating both CD8+ cytotoxic T cells and CD4+ helper T cells.

and attack cancer cells expressing these antigens (Fig. 5). Compared to conventional treatments, this approach is clinically relevant because it represents targeted therapy with potential less damage to healthy tissues. BNT116 is currently being investigated in a Phase I trial (NCT05142189) for dosing, safety profile and preliminary efficacy assessment, and in a Phase II study in combination with cemiplimab as a first-line treatment for patients with advanced or metastatic NSCLC (NCT05557591). The development of BNT116 may represent a significant step in the field of mRNA-based cancer immunotherapies, with the potential to positively revolutionize the treatment of NSCLC. There are however key challenges regarding BNT116 including overcoming the immunosuppressive tumor microenvironment and ensuring durable immune responses through optimized delivery (Chen et al., 2024). Furthermore, larger trials are needed to confirm survival benefits and establish clinical usefulness (Lorentzen et al., 2022).

The development of mRNA-4157 (or V940; oderna and Merck) is a significant advancement in cancer immunotherapy due to its personalized approach. Unlike "off-the-shelf" vaccines that target common antigens, mRNA-4157 is an individualized neoantigen therapy custom-designed for each patient (Weber et al., 2024). The process involves tumor biopsy and DNA sequencing to analyze the unique mutational signature of the tumor and identify specific neoantigens. Neoantigens are novel proteins arising from cancer-specific mutations in the tumor DNA that are not found in healthy cells. Based on the identified neoantigens (up to 34 of them), a synthetic mRNA sequence is designed and produced. This mRNA codes for these specific neoantigens. When the mRNA vaccine is administered to the patient, their cells translate the mRNA into the neoantigens. These neoantigens are then presented on the surface of APCs. This process ultimately activates the T-cells of the patient to specifically recognize and destroy tumor cells expressing these unique neoantigens. mRNA-4157 is often evaluated in combination with immune checkpoint inhibitors, particularly pembrolizumab (PD-1 inhibitor). The rationale is that the mRNA-4157 increases the number of tumor-specific T-cells, whereas the checkpoint inhibitor "releases the brakes" on these T-cells, allowing them to more effectively attack the tumor. mRNA-4157 is currently evaluated, in monotherapy as well as in association with pembrolizumab, in different type of solid tumor including NSCLC (NCT06623422 – Phase III), melanoma (NCT05933577 – Phase III), cutaneous squamous cell carcinoma (NCT06295809 – Phase III). In 2023, mRNA-4157 was granted breakthrough therapy designation by the FDA for adjuvant treatment of patients with high-risk melanoma following complete resection providing evidence that an mRNA-based personalized neoantigen therapy could be advantageous. In summary, mRNA-4157 represents a cutting-edge personalized cancer vaccine approach supported by very promising clinical data, particularly in melanoma. However, optimizing neoantigen selection to maximize immunogenicity remains a major obstacle due to the complex nature of tumor genomics and immune recognition (Katsikis, Ishii, & Schliehe, 2024). Also, scaling up the production of personalized vaccines like mRNA-4157 raises logistical and financial challenges, requiring efficient, rapid manufacturing workflows to deliver patient-specific doses without compromising quality (Singh et al., 2025).

Furthermore, BioNTech in collaboration with Genentech has developed the personalized mRNA-based cancer vaccine cevumeran (BNT122), which can incorporate up to 20 MHC Class I and MHC Class II neoantigens encapsulated in lipoplex nanoparticles. After administration (typically intravenously), the mRNA-lipoplex is taken up by APCs. These APCs translate the mRNA into individual tumor-specific neoantigen proteins and present them on their surface via MHC molecules. This induces the activation and expansion of either cytotoxic T-lymphocytes (CD8+ T cells) or helper T cells (CD4+ T cells) that are specific for the neoantigens. These activated T cells then recognize and target cancer cells expressing the same neoantigens, leading to a selective anti-tumor immune response. BNT122 is currently under investigation in several clinical trials evaluating its efficacy as a monotherapy and in combination with atezolizumab for patients with locally advanced or metastatic solid tumors (NCT03289962 – Phase I), as a first-line treatment in combination with pembrolizumab in patients with previously untreated advanced melanoma (NCT03815058 – Phase II); in association with Nivolumab in participants with urothelial carcinoma (NCT06534983 - Phase II) and in combination with Atezolizumab and mFOLFIRINOX in patients with resected pancreatic ductal adenocarcinoma (NCT05968326 - Phase II). Cevumeran has shown promise in inducing immune responses and delaying recurrence in some cancers, but clinical efficacy is limited by variable patient responses and safety concerns (Lopez et al., 2025; Rojas et al., 2023). These challenges highlight the need for better patient selection and optimized combination therapies to improve outcomes.

Another promising mRNA-based vaccine developed by BioNTech is Individualized Vaccine Against Cancer (IVAC) mutanome, a poly-neoepitopic encoding vaccine that targets various patient-specific mutant epitopes, with potential immunostimulatory and antineoplastic activity (Mutanome draws Genentech deal, 2016). This poly-epitopic mRNA vaccine encodes for 10 potentially immunogenic mutated sequences identified in patients by next generation sequencing. Its mechanism is similar to that of cevumeran (BNT122) discussed before. IVAC mutanome completed a Phase I trial (NCT02035956) in patients with stage III and IV malignant melanoma in which the candidate vaccine exhibited an optimal pharmacological activity and a good safety profile. The therapeutic approach has been combined with checkpoint inhibitors (like anti-PD-1 therapy) to potentially enhance anti-tumor effects, as observed in some patients achieving complete responses. The IVAC mutanome approach is currently broadly referred to as Individualized Neoantigen Specific Therapy (iNeST), with cevumeran (BNT122) being a key candidate under this umbrella. This approach is under investigation for the treatment of a range of solid tumors beyond melanoma, including pancreatic cancer, urothelial carcinoma, and colorectal cancer. Also in this case, challenges persist in optimizing neoantigen selection and enhancing durable clinical responses across heterogeneous tumor types.

A recent innovative approach in cancer immunotherapy is represented by mRNA dendritic cell (DC) vaccines, which combine the specificity of mRNA technology with the potent capacity of dendritic cells to initiate and regulate both innate and adaptive immune response (Kranz et al., 2016; Lee, Yam, & Mao, 2023). In mRNA DC vaccines, dendritic cells are loaded ex vivo with mRNA encoding tumor-specific antigens. This process enables the cells to produce and present these antigens to the immune system upon their reintroduction into the body. Nevertheless, several studies have reported conflicting outcomes regarding the efficacy of the treatment. Indeed, some studies provide evidence that mRNA-based dendritic vaccines can delay disease relapse and enhance survival (Anguille et al., 2017; De Keersmaecker et al., 2020), while others indicate that mRNA-loaded DC vaccines exhibit technology-related limited clinical efficacy (Perez & De Palma, 2019). Among mRNA DC vaccines, TriMixDC refers to a type of DC vaccine which has been extensively studied in the context of melanoma. In line with other DC vaccines, TriMixDC utilizes the own immune cells of the individual to stimulate an anti-tumor response. Specifically, TriMixDC is constituted of monocyte-derived dendritic cells electroporated with mRNA encoding for three immunomodulators, TLR-4, CD40L and CD70. The TriMix components enable the maturation and activation of DC activated once re-introduced into the patient, which is necessary to overcome the immune-suppressive environment of the tumor. Through this mechanism, TriMixDC activates both CD4+ helper T cells and CD8+ cytotoxic T cells, leading to a more robust and sustained immune response against cancer cells. This mRNA DC vaccine is currently being evaluated in a Phase II trial (NCT01302496) in combination with ipilimumab (an anti-CTLA-4 antibody) in patients with previous treated unresectable melanoma. The clinical data obtained demonstrate that TriMixDC-MEL is immunogenic and exhibits an optimal anti-tumor activity. The results are also interesting considering the combination with

ipilimumab. The side effects observed are basically tolerable and often include local skin reactions at the injection site and flu-like symptoms (De Keersmaecker et al., 2020).

Although not yet approved, numerous mRNA-based vaccines are currently evaluated in clinical trials for several infection diseases, including Rabies virus (RABV), Zika virus (ZikV), Herpes simplex virus (HSV), and Epstein-Barr Virus (EBV). RABV is responsible for one of the most lethal zoonotic diseases, constituting one of the major social and economic burdens associated with nearly 60,000 deaths every year (Hampson et al., 2015). Despite great advances, RABV disease treatment still constitutes a challenge because therapies must be started before the appearance of clinical symptoms (Du Pont, Plemper, & Schnell, 2019). Currently, three mRNA vaccines, CV7201 and CV7202 by CureVac and GSK3903133A by GlaxoSmithKline have completed Phase I studies (NCT02241135; NCT03713086). CV7201, the initial formulation developed by CureVac, encloses mRNA encoding for the glycoprotein of the RABV. However, despite a good tolerability, CV7201 failed to elicit an adequate immune response (Alberer et al., 2017). A subsequent mRNA vaccine, CV7202, consisted of mRNA encoding for the same RABV-G antigen. CV7202 utilizes a next-generation LNP formulation to encapsulate and deliver the mRNA. This LNP-based approach has proven to be superior to the protamine-based formulation used in CV7201. CV7202 exhibited good tolerability and elicited an important immune response at very low doses (Aldrich et al., 2021). This is a key factor for a prophylactic vaccine, for which safety and tolerability are essential. The development of CV7202 represents a significant step forward in the field of mRNA vaccines. Its effectiveness in inducing robust and well-tolerated immune responses at low doses underlines the potential of mRNA technology and the critical role of advanced delivery systems including LNPs. However, further clinical development is needed for CV7202 in order to reach the market. GSK3903133A is an investigational self-amplifying mRNA vaccine candidate. Similar to many other advanced mRNA vaccines, GSK3903133A is based on the LNP approach to encapsulate and deliver the self-amplifying mRNA. Differently from conventional mRNA vaccines that deliver a single copy of mRNA per dose, this vaccine contain additional viral genes allowing the replication of the mRNA (Bloom, van den Berg, & Arbuthnot, 2021). This implies that the administration of low initial doses of mRNA can potentially result in a stronger and long-lasting immune response through a sustained and amplified production of the target antigen (in this case, the rabies virus glycoprotein G). The dose sparing, which allows to trigger strong immune responses with lower doses of mRNA, can be also positive for manufacturing efficiency and vaccine supply. GSK3903133A has demonstrated a favorable safety and immunogenicity profile in clinical trials, with neutralizing antibody responses comparable or superior to other rabies vaccines (Wu et al., 2023). Despite these promising results, long-term and real-world effectiveness needs to be assessed.

ZikV, transmitted through infected mosquitos, has received great attention due to its association with severe neurological complications such as Guillain-Barrè syndrome in adults and congenital Zika syndrome in newborns. However, despite great efforts to develop vaccines and therapeutics, to date, no specific treatments or prophylactic agents are available. Moderna is the first pharmaceutical company developing the two vaccine candidates mRNA-1325 and mRNA-1893 for the prevention of ZikV infection. The vaccines contain mRNA sequences that encodes for the structural proteins of the ZikV. In preclinical models, mRNA-1893 showed more immunogenicity compared to mRNA-1325 and also protected non-human primates against a challenge with ZikV (Bloom et al., 2021). This was possibly related to a difference in a single amino acid residue in the E gene, which influenced the production of virus-like particles (Bollman et al., 2023). In Phase I trials (mRNA-1325 - NCT03014089; mRNA-1893- NCT04064905) both vaccines were generally well tolerated, with no serious ADRs. RNA-1325, mRNA-1893 induced a robust ZIKV-specific neutralizing antibodies response at all doses tested (Essink et al., 2023). mRNA-1893 is currently undergoing further safety, tolerability, and reactogenicity evaluation in a Phase II clinical trial (NCT04917861). mRNA-1893, which has received Fast Track Designation (2019) from the FDA, is a promising mRNA vaccine candidate targeting ZikV with ongoing clinical assessment reporting favorable safety and immunogenicity profiles.

Interestingly, mRNA technology advancements resulted in the development of mRNA vaccines also for seasonal influenza. In particular, four vaccines are currently in clinical trials: mRNA-1010, mRNA-1020, mRNA-1030 (NCT04956575; NCT05333289; Moderna), and BNT161 (NCT05052697, BioNTech/Pfizer). mRNA-1010, mRNA-1020 and mRNA-1030 contain mRNAs encoding for the hemagglutinins (HA) of four influenzas strains A/H1N1, A/H3N2, B/Victoria, and B/Yamagata; however, mRNA-1020 and mRNA-1030 are differentiated by the inclusion of mRNAs encoding for the neuraminidases of the respective viruses at different HA: NA ratios (Tian, Deng, & Yang, 2022). Above the three quadrivalent vaccines, only mRNA-1010 exhibited higher immunogenicity for both influenza A and B strains without treatment-related serious adverse events in Phase I/II (NCT05606965; NCT04956575). Further evaluations of mRNA-1010 are ongoing in two Phase III studies (NCT05415462; NCT05566639). The quadrivalent influenza vaccine candidate BNT161 is a modified mRNA encoding for the HA antigen of four influenzas (two A and two B) strains, recommended by the World Health Organization and the FDA's VRBPAC. Following a successful Phase I/II study (NCT05052697), BioNTech and Pfizer are now assessing the efficacy, safety, and immunogenicity profile in a Phase III randomized, controlled trial (NCT05540522). BNT161 is a significant part of BioNTech and Pfizer's efforts to take advantages of mRNA technology for influenza prevention, based on their experience with COVID-19 vaccines. Despite encouraging immunogenicity and safety findings, there are several challenges for the development of mRNA influenza vaccines. The efficacy of these vaccines across different influenza strains, especially influenza B, remains inconsistent. It has been also observed increased reactogenicity compared to traditional vaccines. Moreover, long-term protection data are limited, particularly in elderly and immunocompromised patients. These factors highlight the need for larger, well-powered studies to confirm if mRNA vaccines can outperform commercially available influenza vaccines in effectiveness and population coverage (Soens et al., 2025).

mRNA-based vaccines hold potential applications also in the prevention for HIV infection (Deeks et al., 2015). HIV is a highly and quickly mutable virus. Mutations occurring during the replication, result in resistance to antiretroviral treatment (Clutter et al., 2016), which represent one of the greatest challenges for the development of a preventive vaccine. Of particular interest, iHIVARNA-01 is an investigational therapeutic HIV vaccine designed to induce strong, targeted immune responses against HIV-1 in infected individuals. iHIVARNA-01 is based on in vivo modifications of DCs. It consists of naked mRNA sequences combined with HIVACAT immunogen, which is specific to key HIV regions, and TriMix (CD40L, caTLR4 and CD70), which provides the necessary activation molecules to enhance DCs and T-cell-activation (de Jong et al., 2019). Phase IIa trials (NCT02888756) build upon the safety and reactogenicity findings of Phase I in which iHIVARNA-01 demonstrated an adequate tolerability and safety profile (NCT02413645). The main objective of the treatment with iHIVARNA-01 is to achieve a functional cure for HIV-1 infection. iHIVARNA-01 might complement or potentially replace antiretroviral therapy, thereby improving the care and reducing the cost associated with HIV management. It is important however a further optimization to improve clinical outcomes, considering the modest immunogenicity and transient effects observed on viral markers (de Jong et al., 2019).

Moderna recently developed other vaccine candidates such as mRNA-1608, mRNA-1647, mRNA-1777 and mRNA-1345, for different infection diseases. mRNA-1608 represents a potential innovative therapeutic vaccine targeting HSV2, which is the primary etiological agent of recurrent genital herpes, resulting in recurrent genital lesions and associated complications (Cohen, 2017). mRNA-1608 is a trivalent vaccine that delivers mRNA encoding for specific HSV proteins (gC2,

gD2, and gE2), which are necessary for the generation of antibodies and cell-mediated immunity (T-cell responses). The efficacy and safety of mRNA-1608 are currently under investigation in a Phase I-II trial in patients with recurrent HSV-2 infection (NCT06033261). There is currently no approved preventive vaccine for herpes simplex virus (Dropulic & Cohen, 2012). The marketing of a therapeutic vaccine like mRNA-1608 would address a significant unmet medical need for millions of people affected by recurrent genital herpes, potentially improving their quality of life and reducing transmission. This may be a potential breakthrough in herpes management.

mRNA-1647 is an investigational mRNA-based vaccine directed against CMV consisting of six mRNA sequences, encapsulated in LNP, encoding for glycoprotein B and the pentameric glycoprotein complex antigens. These proteins are crucial for the virus to enter human cells. The clinical evaluation of mRNA-1647 is ongoing in a Phase III clinical trial (NCT05085366), which has provided preliminary promising data regarding the prevention of congenital CMV transmission (Akingbola et al., 2025b). The primary focus for mRNA-1647 is the prevention of primary CMV infection in women of childbearing age to reduce the risk of congenital CMV transmission to their fetuses. Currently, there is no approved vaccine available to prevent CMV infection. Thus, mRNA-1647 might be a significant step forward in addressing this unmet medical need, mainly considering the possibility of severe complications, such as hearing loss and developmental delays, in newborns with congenital CMV. Early-phase trials have demonstrated a robust immunogenicity and a favorable safety profile of mRNA-1647, which is able to elicit strong neutralizing antibodies and T-cell responses. However, long-term efficacy data, especially with regard to the prevention of congenital CMV transmission, are necessary from ongoing Phase III studies (Akingbola et al., 2025b).

4.5. Investigational mRNA-based drugs

The assessment of mRNA-based drugs is currently a primary focus in cancer research (Liu et al., 2023a). Two mRNA-based drugs were developed by Moderna: mRNA-2416 and mRNA-2752. mRNA-2416 is a LNP therapeutic that encodes the OX40 ligand (OX40L), a membrane-bound co-stimulatory protein (member of TNF superfamily) expressed on APCs involved in the promotion of survival and activity of T lymphocytes that target cancer cells (Deng et al., 2022). mRNA-2416 is designed to intratumorally deliver the mRNA, leading to the local production and expression of OX40L on the surface of cells within the tumor microenvironment. The binding of OX40L to OX40 on T cells promotes T cell expansion and activation, enhances memory responses and inhibits regulatory T cell function, thereby boosting the anti-tumor immune response and increasing the tumor cells killing. The mRNA-2416 was evaluated in a Phase I/II study, which however was terminated prematurely due to the failure to meet the efficacy endpoints for either the monotherapy or combination treatment arms (NCT03323398). For this reason, Moderna subsequently decided to focus on a similar but more potent candidate named mRNA-2752. mRNA-2752 is a LNP encapsulating mRNAs, encoding for human OX40L, IL-23, and IL-36γ pro inflammatory cytokines. The synergistic action of these three proteins produces a robust and sustained innate and adaptive immune response against the cancer, including recruitment of immune cells and increased cytolytic activity against tumor cells (Ramalingam et al., 2025). Moreover, the drug is designed for intratumoral injection with the aim of achieving high concentrations of these immune activators within the tumor microenvironment, potentially turning “cold” (immune-excluded) tumors into “hot” (immune-inflamed) ones. Interestingly, a Phase I study (NCT02872025) combining the intratumoral administration of mRNA-2752 with pembrolizumab in 10 women with high-risk ductal carcinoma in situ showed an 80 % objective response rate, including 3 complete responses (Ramalingam et al., 2025). Some patients with complete responses declined surgery and remained disease-free for 1 to 2 years. The treatment was generally well tolerated, with transient flu-like symptoms and local inflammation. mRNA-2752 is also under investigation in a Phase I trial involving participants with histologically confirmed advanced or metastatic solid tumor malignancies or lymphoma (NCT03739931). The investigational treatment is evaluated as monotherapy and in combination with the immune checkpoint inhibitor durvalumab. Larger clinical trials are needed to confirm the long-term clinical benefit and clarify its role in combination with other drugs.

4.6. Approved siRNA-based drugs

Several siRNA-based drugs have received approval, marking significant advances in RNA interference therapeutics. The vast majority of approved siRNA-based drugs, with the exception of patisiran, are conjugated with GalNAc for liver-targeted delivery, enhancing their stability and uptake by hepatocytes.

In 2018 FDA and EMA approved patisiran (Alnylam Pharmaceuticals) for the treatment of hATTR, which as described above is a rare, inherited, and progressive disease caused by a mutation in the TTR gene, which leads to the damage and dysfunction of different tissues and organs. Patisiran is the first-ever RNA interreference therapeutic approved by the FDA. In particular, patisiran is a siRNA that decreases the concentrations of both mutant and wild-type variants of circulating TTR by specifically binding and degrading TTR mRNA. Patisiran operates by recruiting the RISC complex to degrade the TTR gene (Adams et al., 2018). By reducing the amount of TTR protein in the bloodstream, this siRNA helps to decrease the formation of amyloid deposits, thereby alleviating symptoms and potentially halting or reversing disease progression in hATTR patients (clinical trial: APOLLO, NCT03997383) (Adams et al., 2018). Despite its pioneering success, patisiran administration via intravenous infusion every three weeks has generated issues of treatment compliance among patients. Furthermore, patisiran treatment requires vitamin A supplementation due to decreased serum vitamin A levels, indicating systemic metabolic effects that necessitate careful monitoring. While effective in reducing both mutant and wild-type TTR protein, the delivery of patisiran is limited primarily to the liver due to its LNP-based delivery system. This prevents effective targeting of amyloid deposits in other tissues and potentially reduces efficacy in multi-organ disease manifestations (Mansouri et al., 2025).

Alnylam Pharmaceuticals also developed Vutrisiran, which is a siRNA conjugated with GalNAc3 indicated for the treatment of both polyneuropathy associated with hATTR (FDA and EMA approval 2022) and cardiomyopathy associated with hATTR (FDA approval 2024). Like patisiran, vutrisiran binds to TTR mRNA and promotes its degradation, thereby reducing the synthesis of TTR protein. In clinical studies, vutrisiran induced a TTR reduction of 57 % to 97 %, which was sustained for at least 90 days after administration (Habtemariam et al., 2021). Regarding cardiomyopathy associated with hATTR, vutrisiran significantly reduces cardiovascular mortality, cardiovascular hospitalizations, and urgent heart failure visits (Fontana et al., 2025), based on results from the HELIOS-B Phase III clinical trial involving 655 patients (NCT04153149). Regarding polyneuropathy associated with hATTR, vutrisiran improved polyneuropathy symptoms (Adams et al., 2023), as demonstrated in the HELIOS-A Phase III study (NCT03759379). Importantly, the pharmacokinetic profile is similar across different populations (Olatunji et al., 2024). A key difference in its design is the use of a GalNAc conjugate delivery system in contrast to the LNP delivery system used for patisiran. This delivery system, as already discussed, leads to more efficient and targeted delivery to the liver and better pharmacokinetics. Vutrisiran is indeed administered via a subcutaneous injection every three months. This is a better option for many patients as it can be administered at home by a healthcare professional or in some cases by the patient themselves after proper training. However, like patisiran, the selective hepatic delivery may limit the efficacy of vutrisiran in treating multi-organ disease manifestations. Moreover, long-term safety data for vutrisiran are limited, particularly concerning chronic immunogenicity and off-target effects in different patient

populations (Karimi et al., 2024).

The FDA (2019) and EMA (2020) approved givosiran (Alnylam Pharmaceuticals) for the treatment of acute hepatic porphyria (AHP), a rare genetic condition caused by a deficiency in δ-aminolevulinic acid (ALA) synthase (ALAS1), the first rate-limiting enzyme in heme biosynthesis (Anderson et al., 2005). AHP can cause life-threatening attacks affecting the nervous system because the genetic mutation causes the accumulation of the toxic substances ALA and porphobilinogen. Givosiran is a double-stranded siRNA GalNAc conjugate capable of inhibiting ALAS1 mRNA transcription specifically in the liver and consequently reducing the frequency of acute porphyria attacks (Goga & Stoffel, 2022). In the pivotal ENVISION trial (NCT03338816), givosiran (Fig. 6) decreased the annualized attack rate of porphyria by approximately 70–74 % compared to placebo (Balwani et al., 2020; Kuter et al., 2023). After three months of treatment, a significant reduction in urinary ALA and porphobilinogen was further found, with a median reduction from baseline more than 90 %. Givosiran is administered via a subcutaneous injection once a month. The administration is associated with frequent adverse effects, especially injection site reactions and fatigue, which can contribute to treatment discontinuations.

Lumasiran (Alnylam Pharmaceuticals) received approval (FDA and EMA, 2020) for the treatment of primary hyperoxaluria type 1 (PH1), a rare hereditary condition caused by mutations in the alanine-glyoxylate aminotransferase (AGXT) gene, leading to increased oxalate production (McColgan et al., 2023). Patients affected by PH1 can experience nephrocalcinosis, kidney stones, and also renal failure. Lumasiran is a siRNA that targets the hydroxy acid oxidase 1 (HAO1) mRNA, preventing translation into the enzyme glycolate oxidase (GO). The reduction in GO enzyme levels leads to decreased concentration of glyoxylate, resulting in lower urinary and plasma oxalate levels which are implicated in the progression of kidney failure associated with PH1. The approval of the siRNA was based on positive results from Phase III ILLUMINATE trials (ILLUMINATE-A NCT03681184, B NCT03905694, and C NCT04152200), which demonstrated significant reductions in 24-h urinary oxalate excretion and plasma oxalate levels compared to placebo (Garrelfs et al., 2021). The drug showed sustained efficacy and a favorable safety profile in both pediatric and adult patients with PH1. The approval of lumasiran was important because it was the first approved treatment for PH1, whose treatment before this approval consisted of symptomatic treatments including hyperhydration, inhibitors of crystallization, pyridoxine and also renal transplant. However, data of long-term efficacy and safety beyond 2 years are limited. Importantly, the metabolic consequences of chronic HAO1 inhibition, such as potential glycolate accumulation and its acid-base effects, are poorly characterized and require further investigation. Moreover, its high annual cost and uncertain impact on reducing invasive treatments such as transplantation pose significant challenges for widespread clinical use (Gang, Liu, & Mao, 2022).

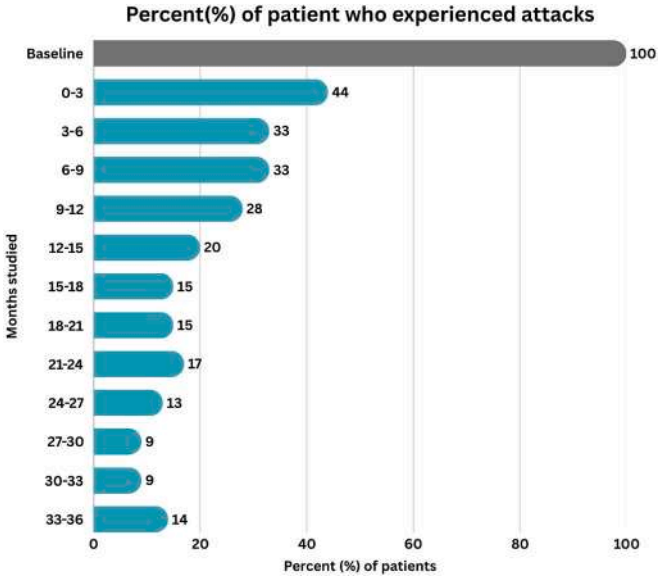

Fig. 6. Givosiran reduces AHP attacks frequency among patients over 36 months.
Data from the final 36-month analysis of the ENVISION study (NCT03338816) indicate that givosiran consistently reduced AHP attack frequency over time (Kuter et al., 2023).

Nedosiran (Dicerna Pharmaceuticals) is a double-stranded siRNA conjugated to GalNAc approved by FDA (2023) to lower urinary oxalate levels in children aged ≥9 years and adults with PH1 and relatively preserved kidney function. Nedosiran binds to and degrades the mRNA encoding for lactate dehydrogenase A (LDHA). By reducing hepatic LDHA, nedosiran blocks the final enzymatic conversion of glyoxylate to oxalate, thereby decreasing hepatic oxalate production and reducing urinary oxalate excretion (Liu et al., 2022). Indeed, clinical findings demonstrate a dose-dependent reduction in urinary oxalate, with effects observed after the first dose and maintained with monthly administration (Syed, 2023). Approval relied on the pivotal Phase II PHYOX2 trial (NCT03847909) and interim data from the Phase III PHYOX3 extension study (NCT04042402), which proved significant and sustained decreases in 24-h urinary oxalate excretion (Baum et al., 2023). Nedosiran has the potential to be effective for other PH subtypes considering its mechanism targeting the final step of oxalate synthesis (Syed, 2023). In this regard, further clinical validation is required before generalizing efficacy and safety across PH variants. It is administered as a once-monthly subcutaneous injection. The most common adverse events are mild injection site reactions, which can include redness, pain, bruising, rash, or dimpling at the injection site. The long-term safety, especially regarding possible metabolic alterations related to LDHA inhibition, is not fully understood.

Inclisiran (Novartis), a GalNAc-conjugated siRNA, was approved by the EMA in 2020 and FDA in 2021, for the treatment of patients with established atherosclerotic CVD who need additional LDL-C lowering as well as for the treatment of Heterozygous Familial Hypercholesterolemia (HeFH). Inclisiran acts by covalently binding to proprotein convertase subtilisin/kexin type 9 (PCSK9) mRNA, thereby inhibiting its synthesis by a RISC-mediated mechanism. PCSK9 normally binds to LDL receptors on the hepatocytes and promotes their degradation. By reducing the amount of PCSK9, inclisiran thus allows more LDL receptors to remain available on hepatocytes (Fig. 7). This, in turn, enhances the clearing of LDL-C from the blood, leading to a significant and sustained reduction in LDL-C levels (NCT05362903) (Lamb, 2021). The tolerability, safety and efficacy profile of inclisiran have been investigated in several clinical trials (NCT04929249; NCT03397121, NCT03814187) demonstrating that inclisiran provides a sustained reduction in LDL levels comparable to two PCSK9-inhibiting monoclonal antibodies, such as alirocumab and evolocumab (Arnold & Koenig, 2022; Rajtar-Salwa et al., 2024). Importantly, whereas other PCSK9-inhibiting monoclonal antibodies directly bind to and neutralize circulating PCSK9, inclisiran acts upstream by preventing the production of PCSK9. This allows for a much less frequent dosing schedule. In this regard, inclisiran is given via a subcutaneous injection using an advantageous dosing schedule. After an initial administration, the second administration is given 3 months later. Subsequent administrations are scheduled every 6 months (Table 1). The long-term safety (beyond 4 years) of inclisiran needs to be assessed, especially in different cardiovascular risk populations. Cost and accessibility remain concerns for broad clinical use. Further studies are necessary to assess potential drug-drug interactions (Mercep et al., 2022).

Fitusiran (Alnylam Pharmaceuticals and Sanofi) is the most recent siRNA approved by FDA (2025). Fitusiran is a siRNA conjugated to GalNAc3 indicated for routine prophylaxis to prevent or reduce bleeding episodes in patients with hemophilia A or B, including those with or

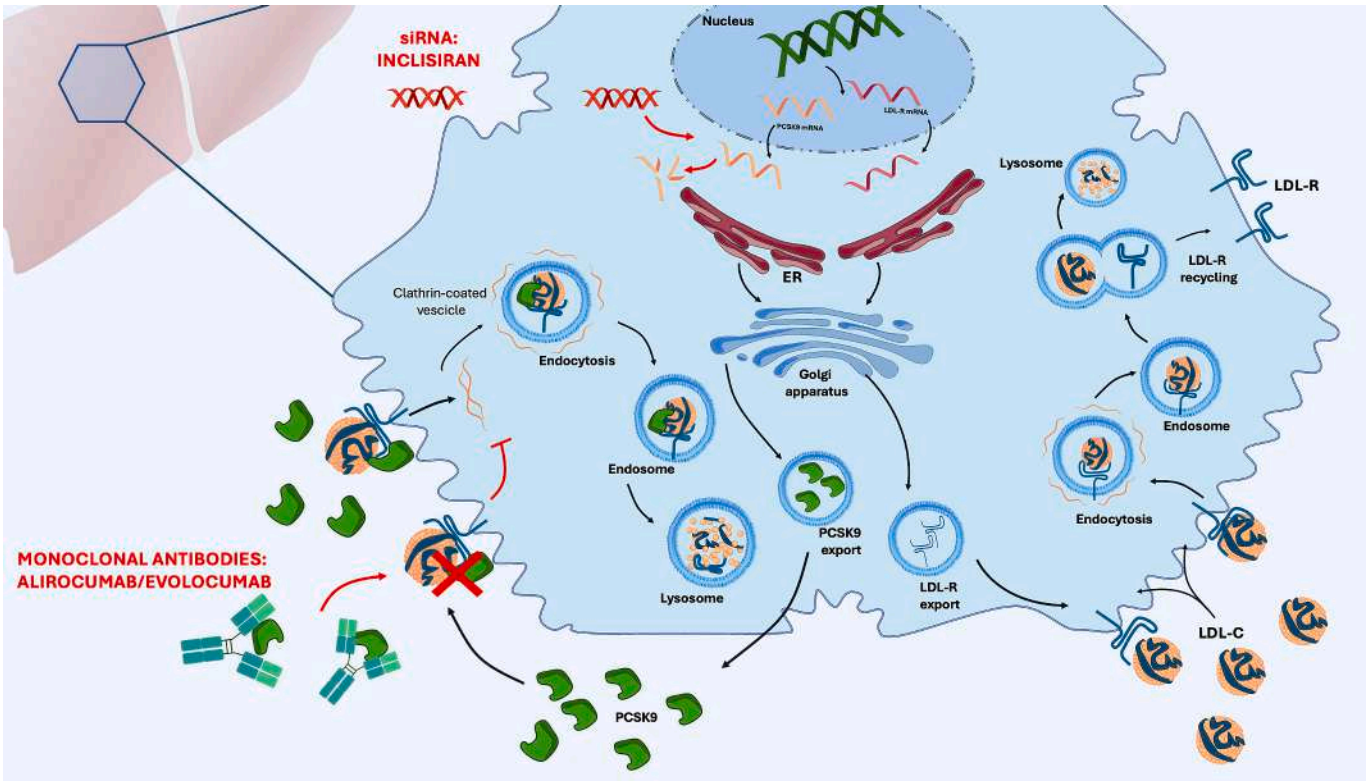

Fig. 7. Comparison of the mechanisms of action of inclisiran and PCSK9-targeting monoclonal antibodies.
Inclisiran, a GalNAc-conjugated siRNA, is selectively taken up by hepatocytes, where it promotes the degradation of PCSK9 mRNA. This leads to sustained suppression of hepatic PCSK9 production, enhanced LDL receptor recycling, and increased clearance of circulating LDL-C. By contrast, PCSK9-targeting monoclonal antibodies, including evolocumab and alirocumab, act extracellularly by binding circulating PCSK9 protein. This prevents PCSK9 from interacting with LDL receptors on hepatocyte surfaces, thereby reducing receptor degradation and preserving receptor density to facilitate LDL clearance.

Table 1
Comparison of the pharmacological profiles of inclisiran and PCSK9-targeting monoclonal antibodies: dosing regimen and LDL-C lowering efficacy (Arnold & Koenig, 2022; Rajtar-Salwa et al., 2024).

Agent	Dosage	Regimen	~ LDL reduction
Evolocumab	140 mg s.c.	Biweekly	~60 %
	420 s.c.	Monthly	~55 %
Alirocumab	75 mg s.c.	Biweekly	~45 %
	150 mg s.c.	Biweekly	~60 %
	300 mg s.c.	Monthly	~ 50 %
Inclisiran	284 mg s.c.	Biannually	~ 50 %

without inhibitors to clotting factors VIII or IX. Fitusiran degrades the mRNA for antithrombin (AT), a natural anticoagulant protein produced in the liver. By lowering AT levels, fitusiran reduces the anticoagulant effect of AT, thus increasing thrombin generation and promoting clot formation. This rebalancing of hemostasis compensates for the deficiency of clotting factors in hemophilia, improving blood clotting and reducing bleeding risk. According to clinical data, fitusiran, administered by subcutaneous injection, consistently lowers plasma AT levels in a dose-dependent manner (Young et al., 2023). Clinically, this translates into a substantial reduction in the annualized bleeding rate in patients with hemophilia A or B (Kenet et al., 2024). The approval indeed was mainly based on findings from the ATLAS Phase III study (NCT03417245), which showed significant reductions in annualized bleeding rates across hemophilia patients with or without inhibitors (Srivastava et al., 2023). Due to its peculiar mechanism of action, the effect of fitusiran on AT levels is sustained, allowing for infrequent subcutaneous administration (e.g., once every two months, or once monthly if needed). Fitusiran is basically well-tolerated with the most adverse effects observed being mild injection site reactions. However, considering the impact of the drug on the coagulation cascade, during the treatment a careful monitoring of AT activity is crucial. This allows for dose adjustments to maintain the desired therapeutic range and minimize the risk of thrombotic complications, which have been observed with persistently low AT levels (Young et al., 2023). Furthermore, long-term safety data are still emerging, and there are some concerns regarding the balance between efficacy and thrombosis risk, especially in real-world heterogeneous patient populations (Lu, Dou, & Gao, 2025).

4.7. *Investigational siRNA-based drugs*

Plozasiran (Arrowhead Pharmaceuticals) has been developed to treat disorders characterized by elevated triglyceride levels, such as FCS, severe hypertriglyceridemia and mixed hyperlipidemia. This drug is a GalNAc-conjugated siRNA designed to inhibit APOC3 mRNA expression, thereby significantly lowering triglycerides levels. APOC3 is a protein mostly produced in the liver involved in triglyceride metabolism. The protein inhibits the breakdown of triglyceride-rich lipoproteins, such as chylomicrons, and also inhibits their hepatic uptake. High levels of APOC3 lead to elevated triglyceride levels in the blood, which can induce serious pathological conditions, including acute pancreatitis, which can be life-threatening, and atherosclerotic CVDs. Phase II Trials have shown promising results for plozasiran, demonstrating its efficacy in reducing triglyceride levels while maintaining an acceptable safety profile (NCT04998201) (Ballantyne et al., 2024). In the pivotal Phase III PALISADE study (NCT05089084), plozasiran induced strong and durable reductions in triglycerides, with a median change from baseline of 80 %. This study also demonstrated an 83 % reduction in the risk of acute pancreatitis after the treatment with the siRNA compared to

placebo (Watts et al., 2025). The most common adverse events were abdominal pain, nasopharyngitis and nausea. More robust data coming from ongoing and future phase III clinical trials will be key to establish the long-term therapeutic value and safety profile of plozasiran. Importantly, the FDA has accepted a new drug application (NDA) for plozasiran in the context of FCS on January 17, 2025. The FDA has also set a Prescription Drug User Fee Act (PDUFA) action date for November 18, 2025.

Olpasiran (Amgen), a siRNA-based drug, is currently evaluated in a Phase III trial for the treatment of atherosclerosis (NCT05581303) and in two Phase I studies for renal (NCT05489614) and hepatic impairments (NCT05481411). This siRNA specifically targets and inhibits lipoprotein (a) [Lp(a)] mRNA, a modified LDL molecule implicated in atherogenesis (Clarke et al., 2009). Olpasiran is also designed to be delivered specifically to hepatocytes using GalNAc conjugate. In the OCEAN(a)-DOSE Phase II trial (NCT04270760), olpasiran demonstrated significant dose-dependent reduction of Lp(a) levels in patients with established atherosclerosis and high baseline Lp(a) levels. These reductions were accompanied by a well-tolerated safety profile with minimal adverse effects reported. Currently, there are no approved therapies for lowering Lp(a) levels. Whereas other lipid-lowering therapies are effective against other cholesterol fractions, Lp(a) remains a significant residual cardiovascular risk factor for many individuals, especially those with established atherosclerotic CVD and elevated Lp(a). Importantly, results from ongoing large-scale phase III trials will be crucial for assessing the correlation between Lp(a) lowering and improved patient outcomes. Olpasiran thus might be a crucial therapeutic option to directly address this unmet medical need.

Zodasiran (ARO-ANG3; Arrowhead Pharmaceuticals) is currently under evaluation for the treatment of homozygous familial hypercholesterolemia and mixed dyslipidemia in phase II clinical trials (NCT05217667). Zodasiran is a hepatocyte-targeted siRNA capable of inhibiting the production of ANGPTL3, which is an inhibitor of the LPL and endothelial lipase (EL). While LPL is responsible for breaking down triglycerides in vLDL and chylomicrons, EL is involved in the metabolism of HDL—C. The reduction in ANGPTL3 levels induced by zodasiran leads consequently to increased activity of LPL and potentially EL. This mechanism in turn produces a significant long-lasting (24 weeks) reductions in triglycerides, LDL-C and non-HDL-C, as demonstrated in a previous Phase IIb clinical trial (NCT04832971) (Rosenson et al., 2024). Zodasiran can offer advantages such as durable gene silencing and infrequent dosing compared to conventional lipid-lowering therapies (Rosenson et al., 2024). However, long-term cardiovascular outcome data and safety evaluation after treatment with zodasiran remain to be elucidated in the phase III clinical trials (YOSEMITE trial; NCT07037771). This siRNA has received orphan drug designation for homozygous familial hypercholesterolemia.

Zilebesiran (Alnylam Pharmaceuticals and Roche) is a pioneering investigational siRNA that might become a long-acting treatment for hypertension, offering a significant potential advantage compared to daily orally administered medications. Zilebesiran targets the renin-angiotensin-system (RAS), a crucial hormonal pathway that plays a central role in regulating blood pressure. Specifically, it is designed with GalNAc conjugate to silence the AGT mRNA and consequently prevents the liver production of AGT. The reduction of AGT circulating levels inhibits the entire RAS cascade including downstream vasoconstrictors like angiotensin I and angiotensin II. This upstream inhibition is a novel and potentially more powerful mechanism (Fig. 8) diverging from the

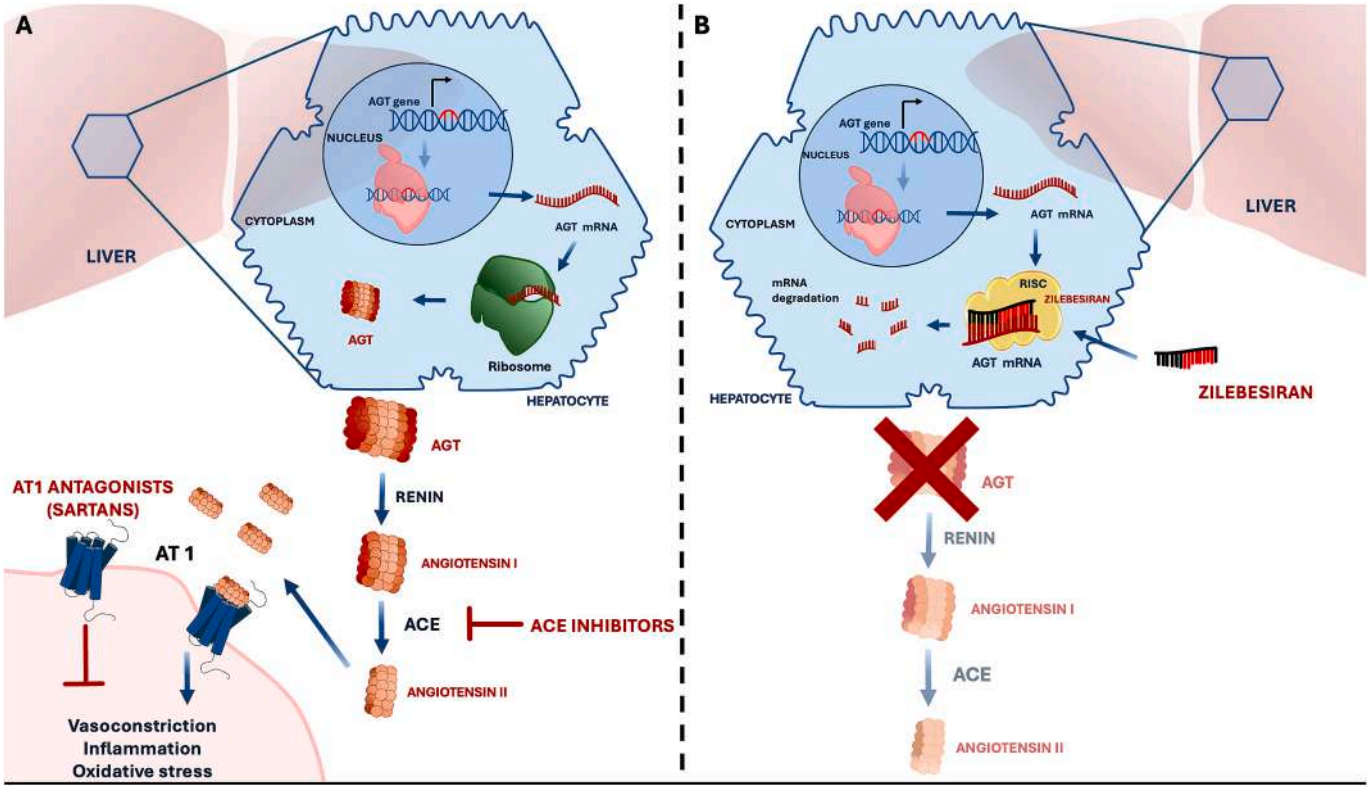

Fig. 8. Mechanism of action of zilebesiran, ACE inhibitors and AT1 antagonists.
A. The mechanism of action of ACE inhibitors and AT1 antagonists involves the inhibition of ACE, which prevents the conversion of angiotensin I to angiotensin II. This inhibition promotes vasodilation and reduces sodium and water retention, ultimately resulting in lower blood pressure. Angiotensin II type 1 receptor antagonists function by blocking AT1 receptors, thereby preventing the binding of Angiotensin II.
B. Zilebesiran is siRNA conjugated to a GalNAc ligand, facilitating its selective uptake by hepatocytes. Once internalized the guide strand directs the RISC to cleave angiotensinogen (AGT) mRNA, effectively silencing its expression in the liver. Consequently, with decreased AGT levels, the renin–angiotensin–aldosterone system is attenuated, leading to diminished production of Angiotensin I and Angiotensin II, which results in lower blood pressure.

traditional mechanisms of RAS inhibitors that block the activity of these downstream components rather than their production (Pu et al., 2025). By targeting AGT upstream in the RAS pathway, zilebesiran may avoid compensatory increases in angiotensin activation observed with traditional RAS inhibitors. Clinical data (Phase I; NCT03934307) demonstrate that a single subcutaneous injection of zilebesiran can induce a significant and sustained reduction in blood pressure for up to six months or even longer, depending on the dose (Desai et al., 2023). Similar results (Bakris et al., 2024) were obtained from the KARDIA-1 Phase II study (NCT04936035). In this respect, the plausible dosing schedule (potentially twice a year) could impressively improve patient adherence to treatment, which can be a challenge in hypertension management. By offering a highly effective and convenient long-acting treatment option, zilebesiran could thus revolutionize hypertension management, particularly for patients showing low adherence to the available treatments. Longer-term clinical outcomes and cardiovascular event decrease findings from the forthcoming large-scale phase III ZENITH trial (NCT07181109) are however needed to totally establish the clinical relevance and safety of zilebesiran in larger populations.

ALN-HSD (Alnylam Pharmaceuticals) is an investigational siRNA developed primarily for the treatment of NASH, which has recently been re-named MASH (Adam et al., 2018). Currently, there are no approved pharmacological treatments for MASH, representing a significant unmet medical need. The mechanism of action of ALN-HSD involves targeting and silencing the mRNA of the hydroxysteroid 17β-dehydrogenase 13 (HSD17B13) gene. The development of ALN-HSD raises after the discovery of a naturally occurring "loss-of-function" genetic variant in the HSD17B13 gene (Ting et al., 2021). Individuals with this variant have a reduced risk of chronic liver disease and progression from simple fatty liver (steatosis) to the more severe inflammation and damage observed in MASH. ALN-HSD aims to mimic this protective genetic effect by reducing the production of the HSD17B13 protein, thereby blocking or reducing liver injury, fibrosis, and inflammation in patients with MASH. Like other Alnylam siRNA therapeutics, ALN-HSD is designed with their Enhanced Stabilization Chemistry Plus (ESC+) GalNAc-conjugate technology, which allows for subcutaneous administration with increased selectivity for hepatocytes. A Phase I randomized, double-blind, placebo-controlled study (NCT04565717) evaluated safety, tolerability, pharmacokinetics, and pharmacodynamics of ALN-HSD in healthy volunteers and patients with confirmed or suspected MASH. The study showed a dose-dependent reduction in hepatic HSD17B13 mRNA by up to 93.4 % at the highest dose and similar reductions in protein levels. ALN-HSD is currently under clinical investigation in a Phase II clinical trial for the treatment of MASH in participants with genetic risk factors (NCT05519475).

JNJ-3989 (GlaxoSmithKline) is an investigational anti-HBV liver-targeted siRNA. JNJ-3989 targets and cleaves all HBV RNA transcripts, thereby leading to a reduction in HBV proteins and antigens (Yuen et al., 2023). Preliminary results from the Phase I trial have demonstrated a favorable clinical profile for JNJ-3989, exhibiting good tolerability and efficacy in reducing HBsAg levels for up to 336 days following the last dose (Yuen et al., 2022b). The REEF-1 Phase IIb (NCT03982186) study showed that combination regimens including JNJ-3989, NAs, and the capsid assembly modulator JNJ-56136379 (JNJ-6379) led to enhanced viral suppression, with greater reductions in HBsAg and other viral markers compared to NAs alone. In particular, GSK has planned to assess JNJ-3989 in a sequential regimen with the investigational antisense ASO bepirovirsen, aiming to achieve higher rates of functional cure in chronic hepatitis B patients.

RG6346 (Dicerna and Roche) is a GalNAc-conjugated double-stranded siRNA targeting the HBV genome S-region. In a first-in-human study (NCT03772249), RG6346 demonstrated favorable pharmacodynamic and safety profiles in both healthy volunteers and patients with chronic HBV, inducing a moderated to marked reduction in hepatitis B surface antigen levels (Gane et al., 2023). RG6346 is now under investigation in a Phase II study as either monotherapy or in conjunction with other investigational and approved HBV treatments (NCT04225715). RG6346 represents a promising drug in the quest for a functional cure for chronic HBV. By targeting viral gene expression, RG6346 can address a key challenge in HBV treatment: the persistent production of HBsAg, which contributes to immune dysfunction and viral persistence.

4.8. Investigational miRNA-based drugs

Targeting miRNAs has demonstrated therapeutic potential mostly in the field of cancer research (Kim & Croce, 2023). Cobomarsen (MRG-106, miRagen Therapeutics) is a LNA-modified oligonucleotide basically designed for the treatment of cutaneous T-cell lymphoma (CTCL), and specifically for the subtype known as *mycosis fungoides* (MF), which is the most prevalent form of CTCL. Cobomarsen works by inhibiting miR-155, whose increase is implicated in the proliferation and survival of MF cancer cells as well as in the regulation of various pathways associated with cell survival (Seto et al., 2018). By inhibiting miR-155, cobomarsen restores the normal expression of genes that are typically suppressed by miR-155 in cancer cells. This induces both a reduction in cell proliferation and an increase in apoptosis of the malignant cells. Cobomarsen was assessed in in vitro and in vivo testing for the non-germinal center B-cell or activated B-cell subtype of diffuse large B-cell lymphoma (ABC-DLBCL) yielding promising results (Anastasiadou et al., 2021). In this regard, cobomarsen might be effective in treating various lymphomas and leukemias where miR-155 is overexpressed. At the clinical level, cobomarsen has been studied in various clinical trials, primarily Phase I and Phase II studies. While some trials have been completed, others have been terminated. For example, a Phase II trial (NCT03713320) comparing cobomarsen to vorinostat for MF was terminated in spite of no safety or efficacy issues. Cobomarsen has also received Orphan Drug Designation (2020) from the FDA for the treatment of T-cell lymphoma, highlighting the need for new treatments for these rare cancers.

MRX-34 (Synlogic) has gained significant attention as a first-in-class miRNA mimic therapy for cancer. MRX-34 acts as a mimic of mir-34a, a miRNA critically involved in tumor suppressor mechanisms that are compromised due to the cumulative mutations associated with carcinogenic processes (Bader, 2012; Hong et al., 2020; Zhang, Liao, & Tang, 2019). The objective of MRX-34 is to restore the levels of miR-34a and to boost its activity. This leads to the downregulation of multiple oncogenes (cancer-promoting genes) that are normally suppressed by miR-34a. These oncogenes include MET, MEK1, PDGFR-α, CDK4/6, BCL2, WNT 1/3, NOTCH1 and CD44 (Abdelaal et al., 2023). MRX-34 was evaluated in two Phase I trials for the treatment of primary liver cancer, other solid tumors and hematologic malignancies (NCT01829971) as well as melanoma (NCT02862145). However, the clinical development of MRX-34 was terminated consequently to serious immune-related adverse events, including four patient deaths. The underlying cause of these toxicities remains incompletely understood. While pharmacodynamic analyses confirmed miR-34a delivery to tumors and modulation of target gene expression (Hong et al., 2020), researchers have debated whether the severe immune toxicities were primarily due to on-target effects of restoring miR-34a, a tumor suppressor microRNA with broad gene regulatory functions (Mockly & Seitz, 2023), or to off-target inflammatory responses induced by the LNP delivery system itself. The LNP delivery system has been implicated in activating innate immune pathways causing systemic inflammation (Verbeke et al., 2022), which might explain the immune-related adverse events observed. Differentiating between these mechanisms is fundamental for progressing miRNA-based therapeutics safely, emphasizing the need for optimized delivery platforms that minimize immunogenicity while preserving therapeutic activity. Despite this, MRX-34 was a pioneering effort in the field of miRNA-based drugs. It demonstrated the potential of microRNA replacement therapy as a novel approach to cancer treatment. Indeed, there are ongoing efforts to ameliorate delivery systems, reduce side effects and explore alternative miR-34 mimics or other therapeutic miRNAs for various cancers (Abdelaal et al., 2023).

TargomiRs consist of targeted bacterial minicells (EnGeneIC Dream Vectors) containing a miR-16-based miRNA mimic. The miR-16 family (miR-16-1, miR-16-2, miR-16-5p) is a well-characterized tumor-suppressive miRNA, whose expression is commonly reduced in various cancer types (Reid et al., 2016). The rationale behind the use of TargomiRs is that restoring the physiological levels of tumor-suppressive miRNAs like miR-16 can inhibit tumor growth. This therapeutic strategy demonstrates promising activity warranting further investigation in subsequent clinical trials. The MesomiR-1 study is a phase I clinical trial (NCT02369198) designed to evaluate the safety and efficacy of TargomiRs as a second- or third-line treatment for patients with recurrent malignant pleural mesothelioma and NSCLC. The MesomiR-1 study successfully established the safety and maximum tolerated dose of TargomiRs in refractory malignant pleural mesothelioma and NSCLC patients, indicating promising early signs of disease control as well as a partial response in a difficult-to-treat patient population. The exclusive immunostimulatory properties of the EnGeneIC Dream Vectors further enhance its therapeutic potential. However, challenges linked to systemic delivery, off-target effects, and the complexity of miRNA regulatory networks exist. In this regard, ongoing advancements in nanotechnology and the potential for combination therapies are paving the way for the clinical application of TargomiRs.

INT-1B3 (InteRNA Technologies) is a synthetic miRNA mimic of the endogenous tumor suppressor miR-193a-3p, formulated in LNPs. INT-1B3 is designed to act as a potent tumor suppressor with a multi-target mechanism of action that encompasses anti-proliferative, anti-migration, anti-metastatic, cell cycle disruption, induction of apoptosis and modulation of the tumor microenvironment that may lead to an enhanced immune response (Telford et al., 2021). INT-1B3 is capable of downregulating multiple oncogenic signaling pathways, such as the PI3K/Akt and Ras/MAPK pathways, and upregulating the tumor-suppressive PTEN pathway. INT-1B3 also modulates the tumor microenvironment by inhibiting CD39/CD73 and downregulating the adenosine-A2A receptor pathway (Duurland et al., 2024). This induces a decrease in immunosuppressive regulatory T cells (FoxP3/Lag3) and monocytic myeloid-derived suppressor cells, increases the maturation of DCs, and induces a T cell-mediated anti-tumor immune response. Promising data were obtained from a phase I trial evaluating the effectiveness of INT-1B3 for the treatment of patients with solid tumors (NCT04675996). However, the phase I study was prematurely terminated due to insufficient founding. The journey from “bench to bedside” for miRNA-based drugs is complex but may revolutionize cancer treatment by offering multi-targeted therapeutic strategies that can overcome drug resistance and improve patient outcomes.

4.9. Approved aptamers

Currently, only two aptamers have been approved despite compelling evidence suggests their therapeutic potential (Keefe et al., 2010). Approved by FDA in 2004, pegaptanib (OSI-Eyetech Pharmaceuticals, Pfizer) was the first aptamer-based therapeutic to reach the market. It is a 28-base RNA aptamer conjugated to polyethylene glycol (PEG) and acts as a selective antagonist of vascular endothelial growth factor (VEGF), specifically targeting the VEGF-165 isoform (Hernandez-Jimenez & de Castro, 2025). Pegaptanib was indicated for the treatment of neovascular (wet) age-related macular degeneration (AMD). By selectively inhibiting VEGF165, pegaptanib effectively reduces pathological ocular neovascularization and vascular permeability, thereby slowing the progression of wet AMD (NCT00858208) and helping to preserve vision (Group, V.I.S.i.O.N.C.T, 2006). However, it was discontinued due to low sales and competition from other anti-VEGF therapies. By 2011, sales significantly declined because more effective treatments, particularly ranibizumab, and the widespread off-label use of the cheaper bevacizumab emerged. The approval of pegaptanib however marked a significant paradigm shift in ocular therapeutics, demonstrating the clinical feasibility of aptamers and serving as a crucial proof-of-concept for RNA-based drugs. Avacincaptad pegol (Astellas Pharma US, Inc.) is the second RNA aptamer conjugated to PEG that achieved FDA approval (2023), marking a significant advancement in the treatment of a different form of AMD. Avacincaptad pegol is a complement C5 protein inhibitor approved for the treatment of geographic atrophy (GA) secondary to AMD. By inhibiting C5, avacincaptad pegol prevents the formation of the active components C5a and C5b and thus reduces complement system overactivity, potentially slowing the progression of retinal atrophy and vision loss in GA patients. The FDA approval of the aptamer was based on positive results from two key Phase III clinical trials, GATHER1 and GATHER2. These trials evaluated the safety and efficacy of monthly intravitreal administration of avacincaptad pegol in patients with GA secondary to AMD. These studies demonstrated that avacincaptad pegol significantly slowed GA progression at 12 months compared to sham treatment. (Danzig et al., 2024). Safety concerns included risks typical of eye injections, such as infection and retinal detachment. Despite these promising results, some challenges remain. The chronic nature of GA requires prolonged treatment and adherence, and long-term safety beyond one year is needed (Patel et al., 2023). Additionally, complement inhibition, while a novel and validated approach, can prone patients to systemic immune-related risks, which warrant careful pharmacovigilance (Abou-Samra et al., 2025).

It is important to consider that both pegaptanib and avacincaptad pegol were developed for different forms of AMD. The observation that both successful therapeutic aptamers target localized ocular diseases and are administered via intravitreal injection is significant. This direct, localized delivery method largely bypasses several systemic challenges characteristic of aptamer drugs, such as quick degradation by nucleases in the bloodstream and the complications of achieving targeted delivery to distant tissues whereas minimizing off-target effects.

4.10. Investigational aptamers

RNA aptamers have also been investigated for cancer treatment due to their capacity to selectively target cancer cells, inhibit cell proliferation or stimulate apoptosis (Kaur et al., 2018). Olaptesed pegol (NOX-A12; TME Pharma N.V.) is a pegylated L-stereoisomer RNA aptmer that binds with high affinity to CXC chemokine ligand 12 (CXCL12). Through this binding, olaptesed pegol neutralizes CXCL12 (Fig. 9). This prevents CXCL12 from interacting with its receptors, CXCR4 and CXCR7, which are involved in various physiological and pathological processes, including tumor growth and metastasis (Huynh et al., 2020). In particular, the CXCL12/CXCR4 axis plays a key role in cancer progression, promoting tumor proliferation, angiogenesis and metastasis. By blocking this axis, olaptesed pegol can potentially limit tumor progression. By neutralizing CXCL12, olaptesed pegol can potentially boost the anti-cancer immune response by inducing anti-cancer immune cells such as killer T-cells (Zboralski et al., 2017). The aptamer has been evaluated in several clinical studies, to assess the safety and efficacy profile in patients with multiple myeloma (NCT01521533 – Phase II), chronic lymphocytic leukemia (NCT01486797 – Phase II), metastatic colorectal and pancreatic cancer (NCT03168139 – Phase II). Currently, it is also under clinical evaluation for the treatment of glioblastoma (NCT04121455 – Phase II). In this respect, it has received FDA clearance (March 2024) for an Investigational New Drug (IND) application and Fast Track designation for the treatment of glioblastoma. A phase I/II trial (GLORIA study; NCT04121455) is evaluating olaptesed pegol in combination with radiotherapy, and sometimes with bevacizumab or pembrolizumab, for newly diagnosed glioblastoma patients. Early results have shown encouraging improvements in overall survival (Giordano et al., 2024). Other large-scale studies are however required to confirm effectiveness across tumor types and evaluate long-term safety profiles.

Rondoraptivon pegol (Guardian Therapeutics), also known as BT200, is a first-in-class PEGylated anti-von Willebrand factor (VWF) aptamer. By binding to VWF, rondoraptivon pegol has a concentration-dependent dual effect. At therapeutic concentrations, the aptamer

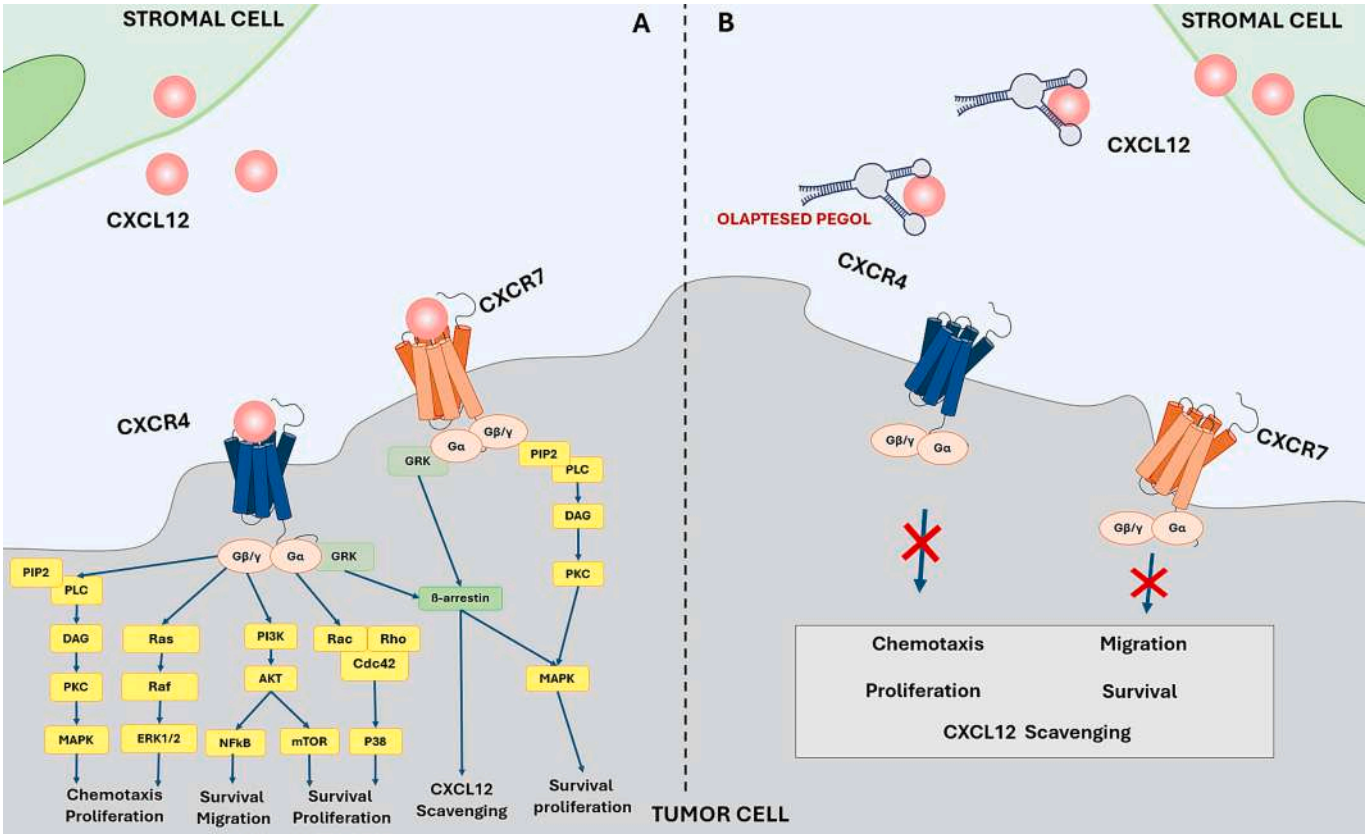

Fig. 9. Mechanism of action of olaptesed pegol.
A. CXCL12 is a chemokine that exert its function through binding to two primary receptors: CXCR4, which plays a significant role in tumor biology, and CXCR7, mainly involved in cell survival and angiogenesis. The activation of the CXCL12-CXCR4/CXCR7 signaling pathway encompasses various roles, including the regulation of tumor cells retention within bone marrow niches, the induction of angiogenesis, tissue repair, metastasis and anti-apoptotic signaling in tumor cells. B. Olaptesed pegol functions by directly binding to CXCL12 and blocking its interaction with CXCR4 and CXCR7. By inhibiting CXCL12, tumor cells are liberated from their protective microenvironment, resulting in an enhanced sensitivity to chemotherapy and immunotherapy as well as exerting anti-angiogenic effects that interfere with tumor neovascularization supported by the CXCL12 pathway.

increases the plasma levels of both VWF and Factor VIII by decreasing their clearance from the bloodstream (Ay et al., 2022). This effect might be particularly beneficial for patients with hereditary bleeding disorders. At higher concentrations, the aptamer can inhibit the binding of VWF to platelet glycoprotein GPIb, which is a key step in platelet adhesion and the formation of blood clots. Rondoraptivon pegol is mainly under clinical investigation for the treatment of hereditary bleeding disorders (von Willebrand disease and hemophilia A; NCT04677803; NCT03370887).

4.11. *Approved CRISPR/Cas-based drugs*

Exagamglogene autotemcel (Vertex Pharmaceuticals and CRISPR Therapeutics) is the first approved gene therapy utilizing CRISPR-Cas9 technology, representing a major advancement in the treatment of genetic blood disorders. It was approved in 2023 by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) and FDA for aged 12 and older for sickle cell disease with recurrent vaso-occlusive crises (VOCs) and in 2024 for patients aged 12 and older with transfusion-dependent beta thalassemia. Exagamglogene autotemcel was also approved in Europe by EMA. Exagamglogene autotemcel employs the ex vivo CRISPR/Cas9 system, which includes a Cas9 enzyme and a gRNA. The gRNA is designed to be complementary to a specific DNA sequence in the BCL11A gene (Cetin et al., 2025). This sequence is localized within the erythroid-specific enhancer region, which regulates the expression of BCL11A. The Cas9 enzyme, guided by the gRNA, works as molecular scissors by making a precise cut at the targeted DNA sequence within the BCL11A gene. This disrupts the function of the BCL11A gene, which basically represses the production of fetal hemoglobin (HbF) (Ma & Qi, 2024). Exagamglogene autotemcel increases thus the production of HbF by reducing BCL11A expression. Considering the sickle cell disease, the increased HbF competes with the abnormal sickle hemoglobin (HbS), reducing the formation of sickle-shaped red blood cells and preventing VOCs. In beta-thalassemia, the increased HbF contributes to compensate for the lack of normal beta-globin, reducing the severity of the disease. The therapy involves harvesting the hematopoietic stem and progenitor cells of the patient, editing them ex vivo, and reinfusing the modified cells after myeloablative conditioning. This ex vivo gene editing approach allows precise editing and quality control before reinfusion, differentiating it basically from gene editing therapies or in vivo administered RNA-based drugs. The approval of the drug was supported by data from pivotal clinical trials including CLIMB-111 (NCT03655678) and CLIMB-131 (NCT04208529), which demonstrated durable increases in HbF, reduction in transfusion dependence, and decreased VOCs events (Frangoul et al., 2024; Locatelli et al., 2024). Despite its breakthrough status of exagamglogene autotemcel, the complexity of manufacture, logistical requirements of cell harvesting and reinfusion, and high treatment costs pose barriers to widespread clinical utilization (Cetin et al., 2025). Long-term studies assessing the persistence of the response, late adverse events, and applicability to broader population are needed.

4.12. Invetigational CRISPR/Cas-based drugs

EDIT-101 (Editas Medicine) is an investigational CRISPR/Cas9 gene-editing therapy developed for the treatment of LCA10, which is as already discussed a rare inherited retinal disease causing blindness. EDIT-101 has been developed to correct the IVS26 mutation in the CEP290 gene, which represents a frequent cause of LCA10 (Maeder et al., 2019). This mutation leads to a faulty splicing of the CEP290 mRNA and production of a non-functional protein. EDIT-101 utilizes CRISPR-Cas9 technology, using two guide RNAs (gRNA-323 and gRNA-64) to target the IVS26 mutation and a Cas9 enzyme to make cuts in the specific sequence of DNA. This gene editing restores normal CEP290 function, improving photoreceptor health and vision (Pierce et al., 2024). Unlike exagamglogene autotemcel, EDIT-101 is delivered directly in vivo as a gene editing therapeutic thorough an adeno-associated virus (AAV5) vector injected subretinally, which target photoreceptor cells. Here, the RNA component (gRNAs) is part of a larger multi-component gene editing system. EDIT-101 is under evaluation in clinical trials, including the BRILLIANCE trial (NCT03872479), which has reported preliminary encouraging results regarding safety and potential for visual improvement (Pierce et al., 2024). EDIT-101 represents the first CRISPR gene-editing therapy administered directly inside the human body for a genetic disease, marking a milestone in gene-editing therapeutics. It can offer hope for patients with LCA10, a condition with no effective treatments currently available.

5. Possible applications of RNA-based drugs: Exploring shared molecular pathways among diseases for drug repurposing

Drug repurposing is a strategy that involves the identification of novel therapeutic applications for drugs that have already received approval or are under advanced clinical investigation. This approach has the potential to reduce the duration of drug development process, decrease associated costs and create new opportunities especially for the treatment of conditions that currently lack effective therapeutic options (Ashburn & Thor, 2004). In this scenario, RNA-based drugs demonstrate significant potential for drug repurposing due to their ability to selectively target specific genetic and molecular pathways across a wide range of diseases. However, it is crucial to emphasize that the current evidence we uniquely provide for many proposed repurposing applications is predominantly based on preclinical findings and biomarker correlations rather than clinical validation. Consequently, this section focuses on hypothesis generation and the exploration of shared molecular pathways among diseases that potentially can ultimately lead to new therapeutic applications for RNA-based drugs.

TargomiRs consist of non-living bacterial nanoparticles that operate as a drug delivery system loaded with miR-16. As previously mentioned, TargomiRs, are currently undergoing evaluation in clinical trials for different cancers; however, the literature supports other potential therapeutic applications of a miR-16 mimic. Indeed, miR-16 is a member of the miR-15/16 family, initially identified as an onco-suppressor miRNA due to its ability to bind to Bcl2 gene, which plays a critical role in the regulation of the antiapoptotic pathway. However, recent evidence supports the possible involvement of miR-16 pathologies beyond cancer. Clinical findings reported by Wang et al. (Wang et al., 2020) demonstrated that miR-16 was found to be downregulated in plasma and PBMCs cells of patients with coronary artery disease (CAD) and its expression was negatively correlated with pro-inflammatory IL-6 levels and positively correlated with anti-inflammatory IL-10 levels. The same group reported a downregulation of miR-16 by using in vitro and in vivo models of atherosclerosis (Liang et al., 2016), which is a risk factor contributing CAD. Importantly, the overexpression of miR-16 inhibited the activation of inflammatory macrophages in atherosclerosis by targeting the programmed cell death 4 (PDCD4). These findings indicate the miR-16 as a biomarker and therapeutic target for atherosclerotic CAD. Thus, TargomiRs might have therapeutic potential for the treatment of CAD. Moreover, growing evidence suggests that miR-16 plays an active role in the pathophysiology of major depressive disorder (MDD). miR-16, which is highly expressed in noradrenergic neurons, directly targets the serotonin transporter (SERT) mRNA, thereby regulating its translation and synthesis (Baudry et al., 2010). This regulatory interaction is highly significant because SERT is the primary transporter responsible for the reuptake of serotonin from the synaptic cleft. By controlling SERT levels, miR-16 directly influences the availability of serotonin for neurotransmission and can influence the therapeutic response to widely used antidepressant drugs such as selective serotonin reuptake inhibitors (SSRI). Preclinical studies have shown that down-regulation of miR-16 in the cerebrospinal fluid and raphe nuclei in rats subjected to a chronic unpredictable mild stress protocol causes de novo SERT protein expression (Baudry et al., 2010). In line with preclinical evidence, clinical findings demonstrated a significant down-regulation of miR-16 in patients with MDD compared to healthy controls (Gheysarzadeh et al., 2018), further supporting the contribution of miR-16 to the therapeutic action of SSRI (Baudry et al., 2010). These evidence highlights the potential of a miR-16 mimic as a novel therapeutic approach in the treatment of MDD. Direct modulation of miR-16 through drugs such as targomiRs might represent a promising new class of therapies for MDD. These RNA-based interventions could target fundamental epigenetic mechanisms, offering a potentially more precise and disease-modifying approach to treatment. Moreover, miR-16 mimic could be strategically combined with traditional antidepressants or other non-pharmacological interventions (e.g., neuromodulation techniques) to achieve synergistic therapeutic effects, potentially overcoming treatment resistance and improving overall outcomes. However, such possible clinical applications remain speculative and require robust preclinical proof and clinical trials with well-defined endpoints. Indeed, despite several preclinical findings link miR-16 to MDD, translating these findings into widespread clinical applications presents significant challenges, including the heterogeneity of MDD, the complexities of CNS delivery, and the need to ensure therapeutic specificity. TargomiRs are administered via intravenous infusion in clinical trials for cancers, resulting in systemic distribution primarily targeting tumor cells. However, for neuropsychiatric conditions such MDD, the intravenous route is not the best choice considering the crossing of the BBB. To address this, alternative delivery approaches aiming to improve CNS bioavailability would be critical. For instance, intranasal administration can offer a non-invasive route that bypasses the BBB via olfactory and trigeminal nerve pathways and has shown promise for delivering RNA-based drugs to the brain (Shah, Lalan, & Barve, 2022). Therefore, whereas current intravenous infusion of TargomiRs supports systemic oncological indications, a possible repurposing for MDD or other CNS disorders would require rigorous exploration and optimization of delivery approaches to ensure adequate CNS delivery and therapeutic efficacy. This represents a significant translational challenge needs to be addressed in future preclinical and clinical studies. Overall, targomiRs represent a promising RNA-based therapeutic platform with proved clinical safety and preliminary antitumor activity; their extension to non-oncological diseases such as CAD and MDD is an intriguing but still largely unproven avenue, which demands rigorous multidisciplinary research addressing delivery challenges, pharmacokinetics, pharmacodynamics, and long-term safety.

Cobomarsen (MRG-106), as previously discussed, acts by inhibiting miR-155, which is commonly found at high levels in blood cancers. The therapeutic potential of cobomarsen extends significantly beyond its initial oncology indications due to the multifaceted biological roles of its target, miR-155. miR-155 has been characterized as a key regulator of immune responses, widely expressed in an array of immune cells, including B cells (Eis et al., 2005), T cells (Haasch et al., 2002), macrophage (O'Connell et al., 2007), and dendritic cells (Rodriguez et al., 2007). The inhibition of miR-155 has demonstrated a "renoprotective effect" (Wang et al., 2025) in drug- or substance-induced Acute Kidney Injury (AKI). Targeting miR-155 and its associated pathways

could potentially recover the process of AKI by altering apoptosis, inflammation and pyroptosis. These preclinical findings provide a mechanistic rationale for repurposing cobomarsen in AKI; nonetheless, clinical data remain lacking, and the translation to human patients requires careful pharmacological and safety evaluation. A recent study by Bala et al. (Bala et al., 2023) evaluated the possible involvement of miR-155 in liver fibrosis by investigating the possible therapeutic application of miR-155 inhibition in a mouse model of liver fibrosis. The findings obtained demonstrated that in vivo treatment with a recombinant adeno-associated virus system expressing anti-miR-155 reduced liver damage and fibrosis and that this effect was mediated via STAT3 signaling (Bala et al., 2023). These findings indicate that cobomarsen, by inhibiting miR-155, could effectively mitigate fibrosis progression; confirmation in human studies, dose optimization, and off-target risk assessment are however critical next steps. Interestingly, compelling preclinical and clinical findings report a pathophysiological role of miR-155 in AD, with a significant role both in Aβ deposition and tau phosphorylation (Liu et al., 2023b). miR-155 was found to be significantly increased in the CSF and extracellular fluid of AD patients (Alexandrov et al., 2012). Higher levels of miR-155 were found in human post-mortem CA1 hippocampal tissue from AD subjects compared to healthy subjects (Readhead et al., 2020). In line with this, miR-155 was detected significantly upregulated in the hippocampus but not the entorhinal cortex of APP/PSEN1 mice (AD model), a difference attributed to elevated microgliosis (Readhead et al., 2020). Moreover, intracerebroventricular infusion of a miR-155 inhibitor significantly alleviated cognitive impairment in AD rat models (Liu et al., 2019). This inhibition attenuated increases in pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and their receptors, as well as apoptotic Caspase-3 upregulation in the hippocampus of AD rats. Considering the limited pharmacotherapies for the treatment of AD (Varadharajan et al., 2023) and the discussed pathophysiological role for miR-155 in AD, the therapeutic potential of cobomarsen for AD could be investigated taking into account either preventive or therapeutic approaches. In this regard, the crossing of the BBB can be a significant obstacle for the delivery of the drug to the CNS. The highly selective permeability of the BBB limits the crossing of most large, charged molecules such as RNA-based drugs including ASOs, and necessitates appropriate delivery strategies. Traditional routes of administration like intrathecal or intracerebroventricular administrations bypass the BBB but imply risks including invasiveness, limited drug distribution within deeper brain regions, and potential adverse effects (Kim et al., 2025). Recent advances have investigated less invasive strategies to improve oligonucleotide CNS delivery. These include, as previously discussed for targomiRs, the intranasal administration that can provide direct nose-to-brain delivery, potentially bypassing the BBB while reducing systemic exposure and adverse effects (Borgonetti & Galeotti, 2021). Moreover, emerging nanoparticle-based delivery systems, receptor-mediated transcytosis targeting (e.g., transferrin receptor), and BBB disruption techniques including focused ultrasound combined with microbubbles are under investigation to ameliorate brain uptake of RNA-based drugs (Naimi et al., 2024; Shirmast et al., 2024; Witten et al., 2024). In spite of these significant advances, translation to clinical practice remains challenging considering the complexity of BBB physiology, potential toxicity, immunogenicity of delivery vectors, and the need of achieving therapeutically effective concentrations in targeted brain regions. Therefore, for oligonucleotide-based therapies like cobomarsen aimed at CNS diseases, overcoming BBB limitations through cutting-edge delivery systems is a fundamental prerequisite for clinical success. It is also important to consider that systemic inhibition of miR-155, which is a master regulator with pleiotropic roles in immune modulation and inflammation, raises safety concerns for chronic, non-malignant conditions. miR-155 is widely expressed in immune cells and orchestrates multiple immune and inflammatory pathways (Vigorito et al., 2013). While its inhibition shows benefit in preclinical models of inflammatory and fibrotic diseases, it may also impair host immune response or dysregulate immune homeostasis (Bala et al., 2016). Off-target and pleiotropic effects represent therefore key challenges in this context, underscoring the need for tissue- and cell-specific delivery to minimize systemic exposure and adverse effects. Clinical translation of cobomarsen for chronic non-cancer indications thus necessitates extensive evaluation of safety, immunotoxicity, genomic stability, and long-term effects to ensure therapeutic viability. Overall, the future of cobomarsen may depends on securing strategic partnerships that can basically capitalize on its potent and multi-pathway mechanism of action, and its valuable Orphan Drug Designation. By strategically exploring its application in new therapeutic areas with high unmet medical needs, cobomarsen has the potential to transition from a halted oncology candidate to a valuable therapeutic in the broader landscape of inflammatory and immune-mediated diseases, realizing its full therapeutic promise.

A possible target for cancer therapy is represented by SOD1, previously reported as the target of the ASO tofersen, developed for the treatment of ALS. SOD1 acts by eliminating superoxide radicals and protecting cells from ROS-mediated damage. However, recent evidence revealed that SOD1 protein levels are overexpressed in different tumors, including pancreatic adenocarcinoma (Crnogorac-Jurcevic et al., 2002), lung adenocarcinoma (Somwar et al., 2011), resistant ovarian cancer (Szenasi et al., 2023) and inflammatory breast cancer (Aird et al., 2012). Szènàsi end collaborators found an increased expression of SOD1 in ovarian cancer associated with a platinum-resistant phenotype. The silencing of SOD1 via an investigational RNAi tool increased cisplatin-mediated cytotoxicity and tumor growth inhibition in female Balb/c mice (Szenasi et al., 2023), suggesting the potential application of SOD 1 RNA therapeutic inhibitors as chemosensitizers in specific platinum-resistant ovarian cancer. Further evidence demonstrated that treatment with an estrogen derivative inhibits SOD1 leading to selective induction of apoptosis in human leukemia cells (Huang et al., 2000). Considering these promising cancer-related preclinical studies, the investigation of the effectiveness of tofersen in inducing apoptosis and inhibiting tumor progression in cancers characterized by SOD1 overexpression is warranted. This exploration should include assessment of potential toxicities related to systemic SOD1 inhibition, and the identification of patient subsets most likely to benefit from such targeted interventions. Tofersen is currently administered via intrathecal injection for ALS patients with SOD1 mutations to bypass the BBB and selectively target motor neurons in the CNS. In the context of cancer therapy, systemic intravenous administration taking advantage of nanoparticle carriers or antibody conjugates could enhance tumor targeting and decrease off-target effects (Wu et al., 2022). Intratumoral administration of tofersen could also be explored for accessible tumors to maximize local drug concentration and at the same time minimizing systemic toxicity (Jiang, Fu, & Shen, 2024). Thus, while SOD1-targeting mechanism of tofersen is promising for the treatment of tumors with SOD1 overexpression, the choice of tumor-specific delivery strategies are crucial in order to obtain a possible therapeutic success.

As already discussed, zilebesiran might be a revolutionary long-acting siRNA for the treatment of hypertension. Zilebesiran targets the RAS, by silencing the AGT mRNA. Overactivation of the RAS is associated with stress-related disorders such a post-traumatic stress disorder (PTSD). The RAS is activated by traumatic stress, with renin release leading to AGT cleavage and subsequent production of angiotensin II. Angiotensin II increases vascular resistance and blood pressure and induces hyperarousal by increasing norepinephrine release and preventing its reuptake. This sympathetic overactivation is a hallmark of PTSD, linking the RAS to PTSD symptoms and cardiovascular disease risk in individuals with PTSD (Seligowski et al., 2021). Clinical findings show that the administration of drugs targeting the RAS, such as angiotensin-converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs), is associated with reduced PTSD symptom severity even after adjustment for covariates (Khoury et al., 2012). Importantly, this effect was specific for these drugs and not observed with other

antihypertensive drugs. In this respect, angiotensin II receptors, particularly the type 1 receptor (AT1), are expressed not only in the cardiovascular system but also in brain regions involved in fear and anxiety processing, such as the amygdala, hippocampus and prefrontal cortex (von Bohlen Und Halbach & Albrecht, 2006). Blocking AT1 receptors with drugs like losartan has shown promise in improving fear extinction in animal models (Marvar et al., 2014) and reducing PTSD symptoms in humans (Shkreli et al., 2020). In summary, the overactivation of the RAS contributes to the neurobiological mechanisms underlying PTSD by mainly inducing sympathetic nervous system overactivation. Considering the pharmacological relevance of zilebesiran, targeting AGT with this siRNA could be an effective therapeutic strategy to treat PTSD and associated cardiovascular comorbidities. Importantly, considering that zilebesiran acts by targeting peripheral hepatic AGT production, its potential efficacy in treating PTSD symptoms would be independent of its ability to cross the BBB. In this regard, therapeutic benefit could be achieved by suppressing of peripheral RAS activity, which indirectly would modulate neurobiological pathways involved in PTSD via decreased sympathetic nervous system activation. Therefore, the subcutaneous administration currently used for zilebesiran is consistent with this peripheral mechanism of action and is likely to be appropriate for a potential repurposing in PTSD, bypassing the issues related to CNS delivery. It is important to emphasize that although the preclinical findings and the few clinical findings supporting the therapeutic potential of zilebesiran in PTSD are promising, a drug repurposing in this context would require extensive multidisciplinary research addressing pharmacokinetics, patient stratification, clinical effectiveness and long-term safety.

Zodasiran (ARO-ANG3), as aforementioned, is a promising siRNA candidate designed to treat of homozygous familial hypercholesterolemia by reducing ANGPTL3 production, which could be considered for the treatment of nephrotic syndrome (NS). Indeed, remarkably increased serum and urinary levels of ANGPTL3 has been observed in patients affected by NS (Wen et al., 2023). Moreover, ANGPTL3 was significantly found elevated in kidney tissues of patients with minimal change nephrotic syndrome (MCNS) and other NS subtypes, correlating with podocyte damage and proteinuria severity (Liu et al., 2015; Wen et al., 2023). At the mechanistic level, ANGPTL3 destabilizes the podocyte cytoskeleton by interacting with integrin β3 on podocytes, activating the FAK/PI3K/Rac1 signaling pathway and regulating α-actinin-4 expression (Liu et al., 2015; Wen et al., 2023). In general, zodasiran may have therapeutic potential for the treatment of ANGPTL3-related diseases (Jiang et al., 2019). Indeed, zodasiran is a hepatocyte-targeted siRNA capable of inhibiting the production of ANGPTL3, which is almost completely produced by the liver and secreted into the bloodstream (Wang & Musunuru, 2019). Given the role of ANGPTL3 in podocyte dysfunction and proteinuria, zodasiran may offer a therapeutic benefit in ANGPTL3-related kidney diseases like NS, although clinical validation in this indication is still required.

6. Conclusions and future perspectives

In conclusion, this review provides a comprehensive, critical, and up-to-date pharmacological analysis of the rapidly evolving field of RNA-based drugs, which have emerged as transformative tools in modern medicine. These therapies represent a true paradigm shift, based on their unique ability to selectively target previously undruggable genetic and molecular targets involved in a wide spectrum of diseases. RNA-based drugs have demonstrated versatility across numerous medical areas, ranging from rare genetic disorders and cancers to infectious diseases, metabolic syndromes, and neurodegenerative conditions, thus opening new, previously inaccessible avenues for therapeutic intervention. Significant progress has been made, including several approved RNA-based drugs that have set the stage for future innovations. However, numerous challenges need to be comprehensively addressed to fully establish their therapeutic potential. Critical challenges include improving the pharmacokinetic stability of RNA molecules, refining delivery systems to target tissues beyond the liver and CNS, decreasing off-target effects, and minimizing immune-related toxicities. Regarding forthcoming challenges and opportunities, the future success of RNA therapeutics will depend on the continuous integration of molecular biology insights, pharmacodynamic optimization, and rigorous clinical validation. Well-designed clinical trials using precise biomarkers and optimized endpoints are essential to establish the long-term safety, efficacy, and durability of treatment with RNA-based drugs, particularly in diverse and complex patient populations. Furthermore, drug repurposing approaches and personalized medicine hold great promise to accelerate clinical translation, lower development costs, and realize the full potential of RNA-based drugs as tailored therapies. Ultimately, this multidisciplinary convergence of scientific disciplines and technological innovations is poised to revolutionize the therapeutic landscape, offering hope for patients with conditions that remain difficult to treat. The continuous evolution of RNA-based drugs stands at the forefront of a new medical era, driving forward the promise of precision medicine and the delivery of more effective, specific, and safer treatments.

B. Following administration, mRNA is internalized by APCs, where it is translated into antigenic proteins that mimic a pathogen-specific antigen, thereby triggering an immune response. Target proteins are degraded into peptides by the proteasome and subsequently transported into the endoplasmic reticulum, where the peptides are loaded onto MHC I molecules. The MHC I-peptide complex is then transported to the cell surface for presentation. Moreover, antigen-expressing cells can release the protein, enabling antigen-presenting cells to process it and present it on MHC II, thereby activating $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. The activation $CD8^+$ T and $CD4^+$ T cells enhances both cellular and humoral immune responses by stimulating macrophages and B cells, as well as promoting antibody production.

CRediT authorship contribution statement

Sebastiano A. Torrisi: Writing – review & editing, Writing – original draft, Conceptualization. **Federica Geraci:** Writing – review & editing, Writing – original draft. **Lidia Diolosà:** Writing – review & editing, Writing – original draft. **Angelina De Luca:** Writing – review & editing. **Luca Falzone:** Writing – review & editing. **Filippo Drago:** Supervision. **Massimo Libra:** Supervision, Funding acquisition. **Gian Marco Leggio:** Writing – review & editing, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT Model 4 in the writing process to correct grammatical errors and improve readability of this manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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