Molecular Therapy
Nucleic Acids
Review

Development, opportunities, and challenges of siRNA nucleic acid drugs

Bowen Xiao,[1] Shaopeng Wang,[2] Yu Pan,[1] Wenjun Zhi,[1,3] Chensheng Gu,[1,3] Tao Guo,[1,3] Jiaqi Zhai,[1] Chenxu Li,[1] Yong Q. Chen,[1,2,3] and Rong Wang[1,2,3]

[1]MOE Medical Basic Research Innovation Center for Gut Microbiota and Chronic Diseases, Wuxi School of Medicine, Jiangnan University, Wuxi 214122, China; [2]Jiangnan University Medical Center, Jiangnan University, Wuxi 214122, China; [3]School of Food Science and Technology, Jiangnan University, Wuxi 214122, China

Small interfering RNA (siRNA) drugs were first proposed in 1999. They have reached the market for administration to patients after more than 20 years of development. The US Food and Drug Administration has approved six siRNA drugs in recent years: patisiran, givosiran, lumasiran, vutrisiran, inclisiran, and nedosiran. siRNA drugs are based on the post-transcriptional gene regulation mechanism of RNA interference. These drugs have gained widespread attention for their effectiveness, low dosage, and low frequency of administration. Theoretically, siRNA drugs have great potential due to their ability to silence almost any target gene. However, drug delivery, especially the extrahepatic one, remains a major challenge. Currently, all approved drugs target the liver. The high blood flow, natural filtration function, and drug delivery methods of the liver overall ensure high efficacy and stability of the drugs themselves. This review summarizes the history of siRNA drug development and the mechanisms of action, with a focus on the drug targets, indications, and key clinical trial results to introduce the status of both marketed drugs and those currently in clinical trials. Additionally, this review provides a brief analysis of several key stages of the commercialization process of siRNA drugs.

INTRODUCTION

The concept of RNA interference (RNAi) was proposed in 1998 by Andrew Fire and Craig Mello. They demonstrated that double-stranded RNA is the causative agent of post-transcriptional gene silencing in *Caenorhabditis elegans*.[1] RNAi was identified in 1972, when scientists noticed that sense and antisense RNA molecules bind together and decrease the production of gene products.[2] The discovery of post-transcriptional gene silencing[3] in plants was made in 1992, and subsequent research was performed in 1994. In 1998, it was shown that sense-antisense hybrids or self-complementary transcripts were efficient inducers of post-transcriptional gene silencing in plants.[4] David Baulcombe discovered small interfering RNA (siRNA) for the first time in plants in 1999 and subsequently demonstrated its ability to selectively silence genes in mammalian cells. In 2001, siRNA was found to induce RNAi in human cells, and in 2003, researchers investigated the therapeutic potential of siRNA in animals. Fire and Mello received the Nobel Prize in Physiology or Medicine in 2006 for their significant contribution to the development of RNAi. Since 2003, multiple companies worldwide have developed drugs using the RNAi mechanism. The first generation of drugs faced frequent setbacks due to immature technology and limited efficacy. Patisiran is the world's first siRNA drug that was successfully launched in 2018.[5]

This article specifically describes the mechanisms of action, advantages, and main obstacles encountered in the development of siRNA drugs. In addition, the six siRNA drugs currently on the market are described, as well as the progress in the development of siRNA drugs by leading companies.

siRNA DRUGS

Formation of siRNA and mechanism of action

siRNA is a negatively charged and double-stranded RNA molecule of 21–23 nt. siRNA exerts its effects through RNAi, allowing selective targeting and high specificity.[6] It expands drug targets to upstream RNAs of functional proteins, regulating the expression of target genes at the post-transcriptional level.

RNAi is a highly conserved phenomenon in evolution, where double-stranded RNA induces an efficient and specific degradation of homologous mRNA.[7] The initiation of RNAi starts with a long double-stranded RNA molecule, known as the precursor of siRNA (pre-siRNA), which has a length of 70–100 nt. siRNA is generated by the cleavage of pre-siRNA by the protease complex Dicer.

The mature siRNA associates with the protein Argonaute-2 (AGO2) to form the RNA-induced silencing complex (RISC). During the formation of RISC, AGO2 disassembles the siRNA into two individual strands: the guide strand and the passenger strand. The passenger strand undergoes degradation, while the guide strand works as a template to form base pairs with the target mRNA. The target mRNA is fragmented by the activity of nucleases inside the RISC, leading to the suppression of gene expression.[8] siRNA can be metaphorically

https://doi.org/10.1016/j.omtn.2024.102437.

Correspondence: Rong Wang, MOE Medical Basic Research Innovation Center for Gut Microbiota and Chronic Diseases, Wuxi School of Medicine, Jiangnan University, Wuxi 214122, China.
E-mail: 8202305007@jiangnan.edu.cn

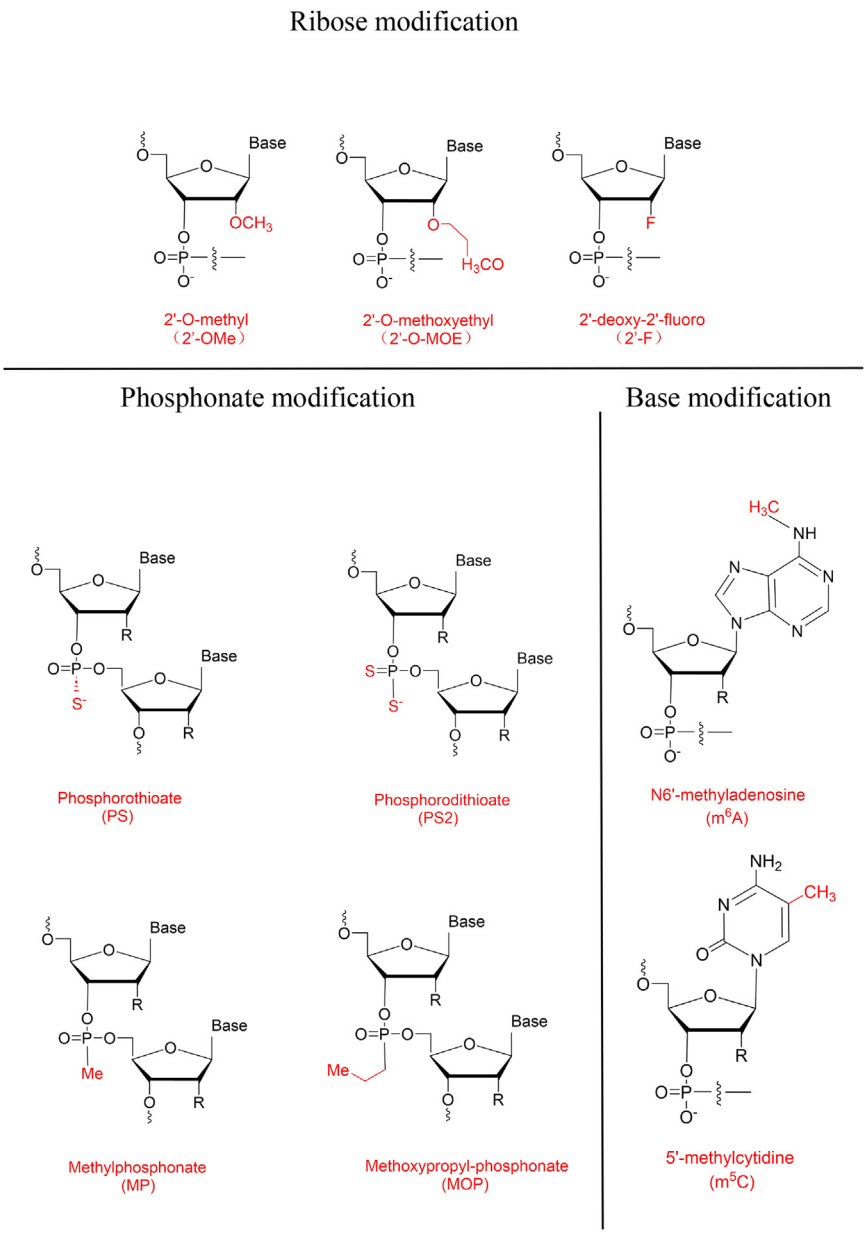

Figure 1. Structures of chemical modifications for siRNA

compared to a pair of genetic scissors that selectively remove disease-related genes to obtain a specific therapeutic effect.

Advantages of siRNA drugs and challenges in development

Most conventional small-molecule drugs act on the proteins and require a higher degree of structural precision compared to siRNA drugs. The development required to obtain conventional small-molecule drugs is significantly difficult and challenging. In contrast, the development of siRNA drugs has become significantly less challenging, with a relatively simpler and more convenient synthesis. Theoretically, siRNA drugs can target and treat any disease with a high degree of target specificity.

siRNA drugs offer several advantages, such as a wide range of ways to administer them, infrequent doses, long-lasting effects in the body, and manageable safety profiles. These drugs have a high efficacy in silencing genes, potentially requiring just one or two doses each year and long-lasting therapeutic effects. However, there are substantial obstacles in advancing their development despite the aforementioned benefits of siRNA drugs over the conventional ones. Unaltered siRNA is effective for a short time in the body and is readily broken down by nucleases. They are easily filtered out by the kidneys due to their small size. In addition, siRNA stimulates the innate immune system, leading to the initiation of an immunological response. Off-target effects and toxicity are significant obstacles to the clinical progress of siRNA drugs.[9]

Scientists have overcome these obstacles after more than 2 decades of study in the field of siRNA drugs by optimizing the chemical modifications of siRNA and the delivery methods. Chemical alterations effectively inhibit the activation of the natural immune response triggered by immunostimulatory siRNA, improve chemical durability and efficacy, and decrease toxicity caused by unwanted targets.[10] These alterations are realized on several components of the nucleotide structure, including the ribose, phosphate backbone, and bases. For example, the replacement of the 2′-OH group on the ribose with a 2′-*O*-methyl (2′-OMe) group, a 2′-*O*-methoxyethyl (2′-MOE) group, or a 2′-deoxy-2′-fluoro (2′-F) group greatly increases the half-life and stability of siRNA,[11] thus improving the accuracy and stability of siRNA binding to the target mRNA and resulting in a higher affinity. Phosphate modifications, including phosphorothioate (PS), increase the resistance of siRNA to nucleases and promote its cellular absorption. Some other phosphate modifications, including phosphorodithioate (PS2), enhance the affinity between RISC and siRNA. Methylphosphonate and methoxypropyl-phosphonate remarkably alleviate hepatotoxicity. Base modifications such as naturally occurring base structures (5-methylcytosine and N6-methyladenine) effectively reduce innate immune recognition while ensuring overall safety (Figure 1). There are many methods for drug delivery, but currently only two delivery approaches have been commercialized: lipid nanoparticles (LNPs) and conjugation-based coupling techniques.

LNPs are chemically synthesized multi-component lipid formulations. They encapsulate siRNA to deliver it to target tissues, protecting the siRNA from nuclease degradation, enhancing tissue penetration, and reducing cytotoxicity. When LNP-siRNA enters the body, the serum protein apolipoprotein E (ApoE) is absorbed onto the surface of the LNP. ApoE has a high affinity for the low-density lipoprotein receptor (LDLR), which is highly expressed on the surface of hepatocytes. Therefore, LNP-siRNA reaches the liver through ApoE-mediated targeting. LNP-siRNA is internalized into hepatocytes and enters the endosomes. The acidic pH environment of the endosomes re-ionizes the ionizable lipids in LNP, which allows the escape of siRNA from the endosomes into the cytoplasm. siRNA exerts its function in the cytoplasm through this method to achieve gene silencing. Patisiran is the first marketed siRNA drug that uses LNP import technology.

Conjugates refer to chemical substances that are fully modified siRNAs coupled with targeting ligands. These conjugates help siRNA find pathways to reach specific cells or tissues in the body. Conjugates operate as a "lock-and-key" system, where the lock is the receptor found on the target cells, and the key is the ligand attached to the siRNA. N-Acetylated galactosamine (GalNAc)-coupled modification is currently the most commonly used siRNA drug delivery system. The siRNA-GalNAc conjugate is a compositionally defined approach for uptake into the liver that increases it into the hepatocytes by ∼10-fold in preclinical models. Cell entry occurs during the internalization and recycling of the asialoglycoprotein receptor (ASGPR). GalNAc is a ligand for ASGPR, which is an endocytic receptor highly and specifically expressed on the surface of hepatocytes. ASGPR-mediated endocytosis effectively transports galactose-derived ligands from the cell surface to the cytoplasm, bringing GalNAc-siRNA conjugates into the cell to form endosomes. A small amount of siRNA escapes into the cytoplasm through the endosomal lipid bilayer to exert its function. Five other siRNA drugs on the market today are GalNAc-modified compounds.

DEVELOPMENT OF siRNA DRUGS

Marketed siRNA drugs

Alnylam Pharmaceuticals has led the development of siRNA-based medicines. Currently, there are six siRNA drugs that have been approved by the US Food and Drug Administration (FDA): patisiran, givosiran, inclisiran, lumasiran, vutrisiran, and nedosiran (Table 1). An introduction to each of these medications is provided in this review.

Patisiran

Patisiran is the first siRNA drug approved for clinical use (2018) in the treatment of hereditary transthyretin-mediated amyloidosis (hATTR) by targeting the transthyretin (TTR) gene.[12] hATTR is a rare genetic disorder characterized by the abnormal accumulation of TTR, leading to the formation of amyloid proteins that accumulate, thus affecting the function of multiple organs. TTR functions as a carrier protein for both thyroid hormone and vitamin A. Mutations lead to the instability of TTR, making it more prone to form deposits. Abnormal TTR proteins accumulate in the body and form amyloid deposits in various tissues and organs, such as the nervous system, heart, and kidneys, affecting their normal structure and function.[13]

Patisiran specifically attaches to and breaks down the TTR mRNA, leading to a decrease in the production of TTR in the liver. It is administered by intravenous injection at 3-week intervals. Corticosteroids, acetaminophen, and antihistamines are administered as premedication to avoid adverse reactions during the administration of patisiran. Patients weighing less than 100 kg are recommended to take a dose of 0.3 mg/kg, while a dose of 30 mg is recommended for patients weighing 100 kg or more (Table 2).

Givosiran

Givosiran is the second siRNA drug approved by the FDA (2019). This drug specifically targets aminolevulinate synthase 1 (ALAS1), which is the first enzyme involved in the synthesis of heme and bilirubin. It catalyzes the synthesis of δ-aminoporphyrin from 5-aminolevulinic acid, an essential process in porphyrin biosynthesis.

Genetic defects in patients with acute hepatic porphyria (AHP) lead to the disruption of certain metabolic pathways, causing abnormal activation of ALAS1. This condition causes an abnormal increase in the synthesis of heme and bilirubin, consequently leading to the disruption of porphyrin metabolism in the body. This results in acute episodes of abdominal pain, neurological symptoms, muscle symptoms, nausea, and vomiting. Givosiran reduces ALAS1 levels in liver cells, lowering the circulating levels of δ-aminolevulinic acid and porphobilinogen, thus treating AHP. The suggested dosage is 2.5 mg/kg of body weight once per month by subcutaneous injection (Table 2).[14]

Lumasiran

Lumasiran was approved by the FDA in November 2020 for the treatment of primary hyperoxaluria type 1 (PH1). Its goal is to lower oxalate levels in the urine and plasma of both children and adults. The medicine silences hydroxy acid oxidase 1 (HAO1) and reduces the presence of glycolate oxidase (GO), which is the enzyme that encodes HAO1. This action inhibits the synthesis of oxalate in the liver and restores its levels to treat PH1.

PH1 is an uncommon hereditary metabolic condition characterized by the excessive synthesis of oxalate, mostly in the liver. Its excessive accumulation causes serious health problems, particularly kidney damage. If an effective intervention is not promptly initiated, then it rapidly progresses to end-stage renal disease, requiring intensive dialysis with a consequent very high mortality rate.[15] The dose of lumasiran depends on the individual's body weight. Patients with a weight above 20 kg are treated with an initial dose of 3 mg/kg once per month for a total of three doses to rapidly achieve a therapeutic level. This is then followed by a maintenance dose of 3 mg/kg administered subcutaneously once every 3 months (Table 2).

Vutrisiran

Vutrisiran is an FDA-approved drug for hATTR on the market from June 2022. It targets the same diseases that are targeted by

Table 1. Recently marketed and clinical stage drugs, as well as the targets of these drugs and associated diseases

Drug	Target	Indication	Phase	NCT no.
Patisiran	TTR	ATTR	commercial	NCT03862807
Givosiran	ALAS1	AHP	commercial	NCT02949830
Lumasiran	HAO1	PH1	commercial	NCT04152200
Vutrisiran	TTR	hATTR	commercial	NCT03759379
Inclisiran	PCSK9	primary hypercholesterolemia (heterozygous familial and non-familial)	commercial	NCT04929249
Nedosiran	LDH	PH1	commercial	NCT04555486
Fitusiran	SERPINC1	hemophilia	3	NCT02035605 NCT02554773
Cemdisiran (ALN-CC5)	C5	complement-mediated diseases	3	NCT02352493 NCT05133531
Belcesiran	A1AT	A1AT deficiency	2	NCT04174118 NCT05146882 NCT04764448
Elebsiran (ALN-HBV02)	unspecified	HBV/HDV	2	NCT03672188
Zilebesiran (ALN-AGT)	AGT	hypertension	2	NCT03934307 NCT04936035
ALN-HSD	HSD17B13	NASH	2	NCT04565717
ALN-APP	APP	CAA/AD	1	NCT05231785
ALN-TTRsc04	TTR	ATTR	1	NCT05661916
ALN-PNP	PNPLA3	NASH	1	NCT05648214 NCT06024408
ALN-KHK	KHK	T2DM	1	NCT05761301
ALN-BCAT	unspecified	hepatocellular carcinoma	1	–
Fazirsiran (ARO-AAT)	A1AT	A1AT deficiency	3	NCT03362242 NCT03945292 NCT05677971
Plozasiran (ARO-APOC3)	APOC3	hypertriglyceridemia	3	NCT03783377 NCT04720534
Zodasiran sodium (ARO-ANG3)	ANGPTL3	ASCVD	2	NCT03747224
GSK4532990	HSD17B13	alcoholic hepatitis/cirrhosis/NASH	2	NCT04202354
JNJ-3989	HBV polymerase and precore/core protein	chronic hepatitis B	2	NCT03365947
ARO-PNPLA3	PNPLA3	NASH	1	NCT04844450
ARO-C3	C3	complement-mediated liver disease/IgA nephropathy	1/2	NCT05083364
ARO-CFB	CFB	complement-mediated diseases	1/2	NCT06209177
ARO-MUC5AC	MUC5AC	mucociliary and inflammatory lung disease	1/2	NCT05292950
ARO-RAGE	RAGE	mucociliary and inflammatory lung indication	1/2	NCT05276570
ARO-MMP7	MMP7	IPF	1/2	NCT05537025
ARO-DUX4	DUX4	FSHD	1/2	NCT06131983
ARO-DM1	DMPK	DM1	1/2	NCT06138743
ARO-SOD1	SOD1	ALS	1	NCT05949294
HZN-457	xanthine dehydrogenase	gout	1	NCT05565768
STP705	TGF-β1/COX-2	isSCC/BCC	2	NCT05422378 NCT04293679

(*Continued on next page*)

Table 1. *Continued*

Drug	Target	Indication	Phase	NCT no.
STP707	TGF-β1/COX-2	hepatic tumor (hepatocellular carcinoma)	1	NCT05037149 NCT05309915
STP122G	factor XI	antithrombotic therapy	1	NCT05844293
Zerlasiran (SLN360)	LPA	LPA-related cardiovascular disease	2	NCT04606602 NCT05537571
SLN-124	TMPRSS6	β-thalassemia/MDS/polycythemia vera	1/2	NCT04559971 NCT04718844 NCT05499013
PH-762	PD-1	melanoma	1	NCT06014086
PH-894	BRD4	solid tumor	1	TrialTroveID-467142
Olpasiran	LPA	ASCVD	3	NCT03626662 NCT04987320 NCT04270760 NCT05581303
Imdusiran (AB-729)	unspecified	HBV	2	TrialTroveID-334421 TrialTroveID-426794

A1AT, α-1-antitrypsin; AD, Alzheimer disease; AGT, angiotensinogen; AHP, acute hepatic porphyria; ALAS1, aminolevulinate synthase 1; ALS, amyotrophic lateral sclerosis; ANGPTL3, angiopoietin-like protein 3; APOC3, apolipoprotein C-III; APP, amyloid precursor protein; ASCVD, atherosclerotic cardiovascular disease; ATTR, transthyretin-mediated amyloidosis; BCC, basal cell carcinoma; 4BRD4, bromodomain-containing protein; C3 and C5, complement components 3 and 5; CAA, cerebral amyloid angiopathy; CFB, complement factor B; COX-2, cyclooxygenase-2; DM1, myotonic dystrophy type 1; DMPK, myotonic dystrophy protein kinase; DUX4, double homeobox 4; FSHD, facioscapulohumeral muscular dystrophy; FXI, factor XI; HAO1, hydroxy acid oxidase 1; hATTR, hereditary transthyretin-mediated amyloidosis; HBV, hepatitis B virus; HDV, hepatitis D virus; HSD17B13, 17-β-hydroxysteroid dehydrogenase 13; IPF, idiopathic pulmonary fibrosis; IsSCC, squamous cell carcinoma *in situ*; KHK, ketohexokinase; LDH, lactate dehydrogenase; LPA, lipoprotein(a); MDS, myelodysplastic syndrome; MMP7, matrix metalloproteinase 7; MUC5AC, mucin 5AC; NASH, non-alcoholic steatohepatitis; PCSK9, proprotein convertase subtilisin/kexin type 9; PD-1, programmed cell death protein 1; PH1, primary hyperoxaluria type 1; PNPLA3, patatin-like phospholipase domain-containing 3; RAGE, receptors for advanced glycation end products; SERPINC1, serpin family C member 1; SOD1, superoxide dismutase 1; T2DM, type 2 diabetes mellitus; TGF-β1, transforming growth factor β1; TMPRSS6, transmembrane protease serine 6; TTR, transthyretin-mediated amyloidosis.

patisiran. However, it is different from patisiran because it utilizes Alnylam's improved stabilization chemistry and third-generation GalNAc-siRNA delivery platform. The prescribed dose for this medication is 25 mg by subcutaneous injection once every 3 months (Table 2).[16]

Inclisiran

Inclisiran was approved by the European Union in December 2020 for the treatment of primary hypercholesterolemia (both heterozygous familial and non-familial) or mixed dyslipidemia, and is the only siRNA medication for lowering cholesterol. This medication specifically inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9), which is an essential enzyme involved in the breakdown of LDL-cholesterol (LDL-C) in the body. PCSK9 inhibits the recycling and reuse process of LDLRs. The decreased PCSK9 expression in the body results in an increase in the number of LDLRs on the surface of liver cells, which bind to more LDL, leading to their removal from the bloodstream.[17] The medicine is administered by subcutaneous injection, initially once at month 0 and again at month 3. Next, the maintenance dose is administered every 6 months, resulting in a total of two injections per year of a volume of 1.5 mL containing 284 mg of the active ingredient (Table 2).

Table 2. Recommended doses and administration frequency of marketed siRNA drugs

Drug	Recommended doses and administration frequency
Patisiran	patients weighing <100 kg are recommended to take a dose of 0.3 mg/kg, while a dose of 30 mg is recommended for patients weighing ≥100 kg; intravenous injection 1× every 3 weeks
Givosiran	suggested dosage is 2.5 mg/kg body weight 1×/month by subcutaneous injection
Lumasiran	dose of lumasiran depends on the individual's body weight; patients weighing >20 kg are treated with an initial dose of 3 mg/kg 1×/month for a total of 3 doses to rapidly achieve the therapeutic level; this is then followed by a maintenance dose of 3 mg/kg administered subcutaneously once every 3 months
Vutrisiran	prescribed dose for this medication is 25 mg by subcutaneous injection once every 3 months
Inclisiran	medicine is administered by subcutaneous injection, initially 1× at month 0 and again at month 3; next, the maintenance dose is administered every 6 months, resulting in a total of 2 injections per year of a volume of 1.5 mL containing 284 mg of the active ingredient
Nedosiran	suggested dose for people weighing ≥50 kg and aged ≥9 years is 160 mg; recommended dose for patients aged ≥12 years and weighing <50 kg is 128 mg; recommended dose for children aged 9–11 years and weighing <50 kg is 3.3 mg/kg; recommended dose for patients weighing ≥50 kg and aged ≥9 years is 160 mg

Nedosiran

Nedosiran was approved in the United States in September 2023 for the treatment of children aged 9 years and older suffering from PH1, as well as adults who have a relatively preserved kidney function. This medication decreases oxalate overproduction by suppressing the expression of liver lactate dehydrogenase (LDH). LDH transforms glyoxylate into oxalate in liver cells, which is the last stage in hepatic oxalate synthesis. Nedosiran is the second siRNA medication authorized for the treatment of PH1, following the approval of lumasiran. However, the target of this medication is different from lumasiran, with a broader range of action. Theoretically, the lowering of LDH potentially ameliorates all subtypes of PH, including PH1, PH2, and PH3.[18] This medication is administered subcutaneously once per month, with a dose according to body weight and age. The recommended dose for patients aged 12 years or older and weighing less than 50 kg is 128 mg, while it is 3.3 mg/kg for children aged 9–11 years and weighingless than 50 kg. The recommended dose for patients weighing 50 kg or more and aged 9 years is 160 mg (Table 2).

Drugs in the clinical trial stage

In addition to the six medications described above that completed the process to become commercially available, there are currently more siRNA drugs undergoing refinement in clinical trials. There are 138 projects listed on ClinicalTrials.gov through July 2024 that involve "siRNA and small interfering RNA." The next step in this review is to examine several leading companies in the field of siRNA drug research and present the drugs that each company is promoting to the market.

Alnylam Pharmaceuticals

Alnylam Pharmaceuticals is at the forefront of the siRNA-based medicine industry. This company produced four of the six siRNA medications currently available for purchase: patisiran, givosiran, lumasiran, and vutrisiran. Alnylam also participated in the creation of inclisiran. The company's drug research and development has expanded to cover a broader range of diseases, such as non-alcoholic steatohepatitis (NASH), Alzheimer disease (AD), and type 2 diabetes mellitus (T2DM). Alnylam is developing more than 10 experimental medications, each at various stages of clinical development. A comprehensive summary of the present progress of each of these medications is presented below.

The medications undergoing phase 3 clinical trials include vutrisiran, fitusiran, and cemdisiran. Vutrisiran is under investigation to evaluate its efficacy and safety in individuals diagnosed with ATTR amyloidosis accompanied by cardiomyopathy. The other two medications are described in Table 1.

Fitusiran specifically targets antithrombin III (ATIII), which is a protein encoded by the serpin family C member 1 (SERPINC1) gene. It inhibits the synthesis of ATIII, thus increasing the coagulation activity. Hemophilia is a hereditary disorder characterized by the lack of specific clotting factors, compromising the coagulation system. Fitusiran utilizes enhanced stabilization chemistry-GalNAc (ESC-GalNAc) conjugation technology for systemic and subcutaneous injection. It is included in nine clinical trials.

The phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT02035605)[19] demonstrated that the drug exerts statistically significant therapeutic effects compared to placebo in both healthy volunteers and patients (ANOVA $p < 0.01$), indicating its potential ability to treat hemophilia. The phase 2 clinical trial (this study was registered at ClinicalTrials.gov: NCT02554773) assessed the long-term safety and tolerability of this drug in patients with severe hemophilia A and B. The results revealed that they are tolerable, being used potentially as a preventive therapy. Four phase 3 clinical trials have concluded, and one is now in progress.

Cemdisiran (ALN-CC5) specifically targets the C5 component of the complement pathway, being used to treat complement-mediated disorders such as paroxysmal nocturnal hemoglobinuria (PNH) and myasthenia gravis. This medication uses Alnylam's GalNAc conjugate delivery system for subcutaneous administration of the doses. C5 activation represents a crucial phase in the complement cascade, leading to the formation of C5a and C5b. Abnormalities in these two components lead to the abnormal synthesis of the membrane attack complex, whose penetration into red blood cells induces hemolysis. This process contributes significantly to the nocturnal hemolysis observed in individuals with PNH. Disruptions in C5a and C5b potentially cause inflammatory response, worsening myasthenia gravis. The medicine is under investigation in a total of 13 clinical trials, with 4 of them already concluded, 6 prematurely interrupted, and 3 still in progress.

A concise overview of the significant trial outcomes is now described. The phase 1/2 clinical trial (this study was registered at ClinicalTrials.gov: NCT02352493)[20] started in January 2015 and aimed to assess the safety and efficacy of the drug in healthy subjects and PNH patients. The initial results of the phase 1 study showed no significant negative adverse events when participants withdrew from the study. Overall, ALN-CC5 was considered safe and well tolerated. The ongoing phase 3 trial (this study was registered at ClinicalTrials.gov: NCT05133531) is assessing the safety and effectiveness of the combination of pozelimab and cemdisiran.

The company is currently performing phase 2 clinical trials for four drugs: belcesiran, elebsiran (ALN-HBV02), zilebesiran (ALN-AGT), and ALN-HSD (Table 1). Most of these medications are designed to treat liver ailments. A concise overview of each medication is summarized below.

Belcesiran specifically targets α-1-antitrypsin (A1AT) primarily for the treatment of liver disease caused by A1AT deficiency (AATD). This hereditary condition is frequently characterized by inadequate or anomalous A1AT levels due to mutations in the SERPINA1 gene, whose accumulation and harm in the liver potentially results in the onset of liver disease. Three clinical trials have been performed for this medication. The phase 1 trial (this study was registered at

ClinicalTrials.gov: NCT04174118)[21] demonstrated that this drug is safe and well tolerated, also inducing a significant and proportional decrease in serum A1AT levels. The phase 2 trial (this study was registered at ClinicalTrials.gov: NCT05146882) was interrupted, while another phase 2 trial (this study was registered at ClinicalTrials.gov: NCT04764448) is under way to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of two different doses of belcesiran in patients.

ALN-HBV02 was created by Alnylam Pharmaceuticals using Vir Biotechnology's ESC+ technology, specifically designed to combat infections caused by the hepatitis B virus (HBV) and hepatitis D virus (HDV). The results of a phase 1/2 clinical trial (this study was registered at ClinicalTrials.gov: NCT03672188)[22] revealed its promising liver safety in both preclinical and clinical investigations. In addition, it induced a dose-dependent reduction of HBV surface antigen (HBsAg) in patients with chronic HBV infection. These results support the use of ALN-HBV02as a potential therapy for HBV.

ALN-AGT is a subcutaneously injected long-acting RNAi therapy that specifically targets angiotensinogen (AGT). Alnylam developed this medication to cure hypertension using its ESC-GalNAc conjugate delivery platform technology.

The medication has successfully concluded three clinical trials. The findings of the phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT03934307)[23] from May 2019 to April 2022 revealed that the continuous administration of the drug induces a remarkable 99% decrease in serum AGT levels and a sustained blood pressure reduction in patients with grade II/III obesity and hypertension. Blood pressure decreased by an average of -27 ± 8 mmHg from the initial measurement to week 24. Finally, the medicine was accepted, with only a few documented negative effects. These results suggest that the pharmacodynamics, efficacy, and safety of ALN-AGT are not affected by body mass index, providing a promising prospect in the treatment of hypertension, even in individuals who are obese. A phase 2 clinical trial (this study was registered at ClinicalTrials.gov: NCT04936035)[24] successfully achieved its primary objective after a 3-month treatment period. It induced a drop in 24-h mean systolic blood pressure of up to 16.7 mmHg when compared to a placebo. The study also met key secondary endpoints, demonstrating a sustained reduction in systolic blood pressure and continued control of hypertension in the sixth month. ALN-AGT was found to be safe and tolerable in subjects diagnosed with mild to moderate hypertension.

ALN-HSD specifically targets 17-β-hydroxysteroid dehydrogenase 13 (HSD17B13),[25] developed by Alnylam in partnership with Regeneron to treat chronic liver disorders and NASH. HSD17B13 is a liver-specific ketone steroid reductase enzyme involved in hormone and lipid metabolism. This medication completed two clinical trials. The phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT04565717) demonstrated promising outcomes, indicating a satisfactory safety and tolerability. Moreover, an evident decrease in liver HSD17B13 mRNA expression was observed. There are other plans in progress to start a phase 3 clinical trial to assess the effectiveness of the medicine in patients with NASH.

Apart from the medications listed above that are in phase 2/3, the company is currently performing phase 1 research on five other drugs: ALN-APP, ALN-TTRsc04, ALN-PNP, ALN-KHK, and ALN-BCAT (Table 1).

ALN-APP is an RNAi therapeutic agent in the target of the amyloid precursor protein (APP)[26] for the treatment of cerebral amyloid angiopathy (CAA) associated with cerebral hemorrhage and AD. APP is the precursor of the β-amyloid protein, and both disorders are characterized by the presence of amyloid plaques. The medicine is undergoing phase 1 clinical research (this study was registered at ClinicalTrials.gov: NCT05231785) to assess its safety, tolerability, pharmacokinetics, and pharmacodynamics after intrathecal administration to adults with early-onset AD.

ALN-TTRsc04 is a subcutaneous RNAi therapy created by Alnylam using its IKARIA platform to treat ATTR amyloidosis by targeting TTR.[27] This drug is currently under phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT05661916).

ALN-PNP targets the patatin-like phospholipase domain-containing 3 (PNPLA3)[28] gene, developed to treat NASH. The drug is being tested in two phase 1 clinical trials (these studies were registered at ClinicalTrials.gov: NCT05648214 and NCT06024408) to evaluate its safety, tolerability, and pharmacokinetics in healthy adult participants, as well as in patients with non-alcoholic fatty liver disease (NAFLD) and patients with genetic risk factors for PNPLA3.

ALN-KHK specifically targets ketohexokinase (KHK),[29] created by Alnylam using its ESC+GalNAc conjugation technology for the treatment of T2DM, since KHK is involved in glucose metabolism. The drug is currently in a phase 1/2 clinical trial (this study was registered at ClinicalTrials.gov: NCT05761301) to assess the safety, tolerability, effectiveness, pharmacokinetics, and pharmacodynamics of a single dose of ALN-KHK in overweight to obese adult volunteers with no other health problems. Additionally, the trial aims to evaluate the safety, tolerability, effectiveness, and pharmacodynamics of multiple doses of ALN-KHK in obese patients with T2DM.

ALN-BCAT specifically targets the WNT pathway[30] to treat hepatocellular carcinoma. The aberrant activation of the WNT pathway is strongly associated with tumor progression. This activation results in an excessive increase in cell division, disruption of normal cell orientation, tissue organization, and a transformation into mesenchymal cells. This also gives tumor cells greater invasiveness and metastatic potential. The medication is now ready for phase 1 clinical trials.

Arrowhead Pharmaceuticals

Arrowhead Pharmaceuticals is a firm specializing in the study and development of RNAi therapeutics, aiming to increase the quality

of life of patients and address many serious chronic diseases through the use of cutting-edge RNA therapies. Their drug development includes a wide range of fields, such as liver, cardiovascular, and motor neuron diseases, among others. The company is developing approximately 10 medications at different stages. Two medications are in phase 3 clinical trials, 3 drugs in phase 2, and 10 drugs in phase 1. Below is an overview of the specific targets, disorders that are involved, and the publicly accessible clinical trial outcomes for each medication.

Fazirsiran (ARO-AAT) and plozasiran (ARO-APOC3) (Table 1) are drugs currently in phase 3 clinical trials. ARO-AAT[31] is currently in the phase 3 stage of clinical testing to address liver diseases linked to AATD, which is a rare genetic disorder severely impairing the liver and lungs of affected individuals. ARO-AAT reduces the production of abnormal A1AT protein in the liver, responsible for progressive liver disease in AATD patients. This medicine was evaluated in four clinical trials. The phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT03362242) was performed from March to October 2018 and demonstrated that it was well tolerated by healthy adult volunteers at all tested doses (up to 300 mg). The drug was administered three times every 28 days, with no occurrences of death or serious (SAE) or mild adverse events. Both single and multiple doses of ARO-AAT effectively decreased serum AAT levels. The phase 2 clinical trial (this study was registered at ClinicalTrials.gov: NCT03945292) showed that the medication effectively decreases A1AT levels in the liver and that its reduction in the serum depends on the dosage. In addition, the medicine showed a decrease in liver inflammation, as assessed by histology. The trial demonstrated good tolerability, with no adverse events related to the study medication. Currently, a phase 3 study (this study was registered at ClinicalTrials.gov: NCT05677971) is under way to evaluate the efficacy of ARO-AAT in the treatment of liver diseases associated with AATD. The study specifically focuses on patients with METAVIR1 fibrosis stages F2–F4.

ARO-APOC3[32] is used to treat hypertriglyceridemia by lowering the synthesis of apolipoprotein C-III (APOC3). The drug is involved in 11 clinical trials to date. The results from the phase 1/2 trial (this study was registered at ClinicalTrials.gov: NCT03783377) performed in March 2019 indicate that the subcutaneous injection of ARO-APOC3 resulted in APOC3 reduction and changes in key lipid parameters in patients with familial chylomicronemia syndrome (FCS) compared to non-FCS patients. The safety parameters in FCS patients were similar to those in non-FCS patients, suggesting that ARO-APOC3 may be a promising RNAi therapeutic drug. The results of the phase 2 trial SHASTA-2 (this study was registered at ClinicalTrials.gov: NCT04720534)[33] on subjects with severe hypertriglyceridemia and baseline triglycerides (TGs) greater than 500 mg/dL revealed that the treatment with 10, 25, and 50 mg of ARO-APOC3 at the 16-week time point consistently reduced APOC3 by 87%, TGs by 86%, and non-high-density lipoprotein-cholesterol (non-HDL-C) by 45%, and increased HDL-C by 99%. The phase 3 trials are still in progress.

Arrowhead Pharmaceuticals is currently performing phase 2 clinical trials using three drugs: zodasiran sodium (ARO-ANG3), GSK4532990, and JNJ-3989 (Table 1).

ARO-ANG3[34] is specifically formulated to decrease the synthesis of angiopoietin-like protein 3 (ANGPTL3). This medication was used in six clinical trials. A completed phase 1/2 trial (this study was registered at ClinicalTrials.gov: NCT03747224) assessed the safety of ARO-ANG3 (primary objective), its pharmacokinetics (in healthy participants), and its pharmacodynamics (secondary objective). ARO-ANG3 is well tolerated, with similar adverse events in both active and placebo cohorts. ARO-ANG3 was quickly and consistently absorbed into the bloodstream of healthy individuals, reaching its maximum concentration between 6.0 and 10.5 h. It was then cleared from the plasma in 24–48 h, with an average duration of 3.9–6.6 h. ARO-ANG3 treatment in healthy participants resulted in a reduction of ANGPTL3 levels by 45%–78% on average at day 85 after administration. The three highest doses of the medication led to a reduction in TG concentrations (median −34% to −54%) and non-HDL-C levels (mean −18% to −29%) (exploratory endpoints). These first studies provide evidence that ANGPTL3 could be a promising target for treating atherosclerotic cardiovascular disease (ASCVD), and indeed there is now the intention to perform a phase 3 trial.

GSK4532990, formerly called ARO-HSD, specifically acts on HSD17B13, which is a component of the hydroxysteroid dehydrogenase family responsible for the metabolism of hormones, fatty acids, and bile acids. The silencing of HSD17B13 decreases the occurrence of alcoholic hepatitis, cirrhosis, and NASH as revealed by published human genetic data. The results of a phase 1/2 trial (this study was registered at ClinicalTrials.gov: NCT04202354)[35] demonstrated that the drug was well tolerated at doses less than or equal to 200 mg. Short-term treatment reduces liver HSD17B13 mRNA and protein levels, together with a decrease in ALT.

JNJ-3989 was previously known as ARO-HBV. The medication was developed by Arrowhead Pharmaceuticals in collaboration with Pfizer as a potential cure for patients with chronic HBV infection alongside other direct-acting antiviral medicines. It is administered by subcutaneous injection to suppress all gene products of the HBV, by intervening at an earlier stage in the reverse transcription process. The results of a phase 1/2 trial (this study was registered at ClinicalTrials.gov: NCT03365947)[36] showed that JNJ-3989 effectively reduced all viral indicators, especially HBsAg, compared to other markers. Hepatitis B e antigen-positive ($HBeAg^+$) patients reported a greater reduction in HBsAg compared to $HBeAg^-$ patients. The decrease in HBeAg, hepatitis B core-related antigen, and HBV RNA is typically linked to the decrease in HBsAg. These findings are confirmed in a more extensive phase 2b trial.

The following medications are undergoing phase 1 clinical trials in Arrowhead Pharmaceuticals' pipeline (Table 1): ARO-PNPLA3, ARO-C3, ARO-CFB, ARO-MUC5AC, ARO-RAGE, ARO-MMP7,

ARO-DUX4, ARO-DM1, ARO-SOD1, and HZN-457. ARO-PNPLA3,[37] previously known as JNJ-75220795, is formulated to reduce the expression of PNPLA3 in the liver. PNPLA3 is present in the liver of patients carrying the common I148M mutation that promotes fat accumulation and injury. A phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT04844450) showed promising results after the administration of a single dose of ARO-PNPLA3, with no SAEs, no significant increase in TGs or LDL-C during treatment, and a dose-dependent average reduction of 40% in liver fat in patients with the homozygous I148M mutation.

ARO-C3[38] reduces the synthesis of complement component 3 (C3), thus working as a potential therapeutic strategy for many complement-mediated disorders, including complement-mediated liver disease and immunoglobulin A nephropathy. A phase 1/2 clinical trial (this study was registered at ClinicalTrials.gov: NCT05083364) is under way, and the results indicate no SAEs or trial interruption due to adverse events. A mean reduction of 88% in C3 levels was consistently observed during the treatment period, providing strong evidence that the administration every 3 months or less frequently is potentially safe.

ARO-CFB decreases the amount of complement factor B (CFB)[39] produced in the liver. It is a regulator in the amplification of the alternative complement pathway, being a potential target for treating kidney disorders, as well as disorders in other parts of the body caused by complement activation. A phase 1/2a dose-escalation trial (this study was registered at ClinicalTrials.gov: NCT06209177) is ongoing, aiming to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of both single and multiple doses of ARO-CFB. This study is being performed on adult healthy volunteers and adult patients who have complement-mediated kidney disease.

The objective of ARO-MUC5AC is to decrease the synthesis of mucin 5AC (MUC5AC)[40] in the treatment of different obstructive and inflammatory lung diseases affecting the mucosal lining. The medicine is being used in two clinical trials. One of the phase 1/2a trials (this study was registered at ClinicalTrials.gov: NCT05292950) aims to evaluate the impact of ARO-MUC5AC on healthy subjects and patients with asthma.

ARO-RAGE reduces the synthesis of receptors for advanced glycation end products (RAGE)[41] to cure different obstructive and inflammatory lung diseases affecting the mucosa. The medication was used in three clinical trials. The phase 1/2 trial (this study was registered at ClinicalTrials.gov: NCT05276570) was completed and showed that ARO-RAGE induced a knockdown of the target gene of up to 90% after a single inhalation dose, with a maximum of 95%. The mid-term results demonstrated that the amount of soluble RAGE in bronchoalveolar lavage fluid and serum decreased in a dose-dependent manner. This indicates that both healthy volunteers and patients with asthma tolerated the treatment well.

ARO-MMP7 decreases the production of matrix metalloproteinase 7 (MMP7),[42] thus being a potential treatment to cure idiopathic pulmonary fibrosis (IPF). The medicine is under phase 1/2a research (this study was registered at ClinicalTrials.gov: NCT05537025) to assess the effect of ARO-MMP7 solution by inhalation on both healthy volunteers and patients with IPF.

ARO-DUX4 targets the gene encoding the human double homeobox 4 (DUX4)[43] protein as a potential therapy for patients with facioscapulohumeral muscular dystrophy (FSHD). This drug is entering phase 1/2a trial (this study was registered at ClinicalTrials.gov: NCT06131983) to evaluate its safety, tolerability, pharmacokinetics, and pharmacodynamics in adult patients diagnosed with FSHD type 1.

ARO-DM1 decreases the expression of the myotonic dystrophy protein kinase (DMPK)[44] gene as a potential treatment strategy for myotonic dystrophy type 1 (DM1). A phase 1/2a dose-escalation trial (this study was registered at ClinicalTrials.gov: NCT06138743) is under way to assess its safety, tolerability, pharmacokinetics, and pharmacodynamics in individuals with DM1 aged between 18 and 65 years.

ARO-SOD1 decreases the production of superoxide dismutase 1 (SOD1)[45] in the central nervous system, being a potential treatment strategy for patients with amyotrophic lateral sclerosis (ALS) caused by SOD1 mutations. The medication is under a phase 1 randomized, placebo-controlled, dose-escalation study (this study was registered at ClinicalTrials.gov: NCT05949294) to assess its safety, tolerability, pharmacokinetics, and pharmacodynamics in adult patients with ALS. The patients involved in the study have an SOD1 mutation that is considered to be the cause of ALS.

HZN-457 is modified with GalNAc to specifically target and suppress xanthine dehydrogenase[46] activity in the liver to treat gout. The medicine has successfully concluded a phase I trial (this study was registered at ClinicalTrials.gov: NCT05565768) involving randomized, placebo-controlled, single ascending dose (SAD) research. The purpose of the study was to assess its safety, tolerability, pharmacokinetics, and pharmacodynamics in a group of healthy volunteers. Nevertheless, the outcomes of this experiment have not yet been revealed.

Sirnaomics

Sirnaomics is a biopharmaceutical company specializing in the development of more than 10 siRNA medicines. Of these, three are undergoing phase 1/2 clinical trials (Table 1), while the remaining are in the investigational new drug application stage or in the preclinical development phase. A concise overview of the three drugs under clinical trials is presented below.

STP705[47] is the primary product of Sirnaomics, working as a dual inhibitor of transforming growth factor β1 (TGF-β1) and cyclooxygenase-2 (COX-2), both viable targets for the development of cancer and fibrosis therapy. STP705 is involved in three clinical trials: an advanced clinical study for *in situ* squamous cell carcinoma,

the last stage of phase 2 for basal cell carcinoma, and the final stage of phase 1 for fat remodeling. Furthermore, STP705 has been designated as an orphan drug for the treatment of cholangiocarcinoma and primary sclerosing cholangitis.

So far, STP705 is involved in 11 clinical investigations. The objective of a phase 1 trial (this study was registered at ClinicalTrials.gov: NCT05422378) is to evaluate its safety and tolerability after subcutaneous injections in adult participants who underwent abdominoplasty. The trial procedure consisted of three treatment cycles separated by a 28-day interval and consisting of seven subcutaneous injections of 120, 240, or 360 μg (in 0.5 mL or 1.0 mL per injection) as well as 1.0 mL placebo. Patients were randomly divided in a double-blind manner and eight subjects aged between 18 and 65 years received injections in seven areas of 1 cm^2 each on the lower abdomen. They were subsequently monitored on days 2 and 7 after the surgery. The results revealed that STP705 was well tolerated at all administered dosages, concentrations, and volumes, demonstrating exceptional safety, with few instances of local skin responses.

A phase 1/2 clinical trial (this study was registered at ClinicalTrials.gov: NCT04293679)[48] that was concluded in October 2020 assessed the safety and efficacy of STP705 administered by intralesional injection in adult patients with *in situ* cutaneous squamous cell carcinoma. The main objective was to assess the percentage of patients who achieved complete histological clearance. Twenty-five patients were treated with STP705, and 19 of them (76%) achieved histological clearance, while 80% and 100% of patients achieved histological clearance in the 30-g treatment group and the 60-g treatment group. These findings revealed that STP705 is a non-invasive, secure, and effective therapy for cutaneous *in situ* squamous cell carcinoma. The suggested doses for future investigations are 30 μg each treatment and 60 μg each treatment.

The company is in the first phases of developing its second significant product, STP707, which is an inhibitor of TGF-β1 and COX-2[49] and intravenously administered. It uses the company's unique polypeptide nanoparticle as a delivery platform for RNAi-based therapy. The medication is being used in two phase 1 clinical trials to assess the safety and effectiveness of intravenous STP707 administration in patients who have advanced/metastatic or surgically unresectable solid tumors (this study was registered at ClinicalTrials.gov: NCT05037149). Initial findings have revealed very encouraging clinical prospects. A separate phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT05309915) is being performed to assess the safety, tolerability, and pharmacokinetics of the intravenously delivered increasing dosages in healthy individuals.

STP122G is in the early stages of development as an anticoagulant in antithrombotic therapy, such as for stroke patients and patients undergoing orthopedic surgery. Leveraging Sirnaomics' proprietary GalAhead platform delivers drugs using GalNAc-siRNA conjugates. This drug specifically targets factor XI (FXI),[50] which is a plasma glycoprotein that is mostly produced in the liver and is involved in maintaining coagulation stability and amplification. A phase 1 trial (this study was registered at ClinicalTrials.gov: NCT05844293) was performed in May 2023 to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of subcutaneous FXI-GalNAc-siRNA SAD in healthy individuals. Part of the trial has been completed, and the safety data demonstrated no dose-limiting toxicity or SAEs; therefore, the study will proceed to the next dose cohort.

Silence Therapeutics

Silence Therapeutics is a firm specializing in developing RNAi medications using a specific delivery system called mRNAi GOLD. The drug targets are in the liver because this organ expresses nearly half of the 30,000 human genes, including thousands of genes associated with diseases. This platform's delivery mechanism in the liver is highly precise, potentially allowing the cure of any condition with a high gene expression in the liver. Additionally, unintended adverse reactions are prevented by its ability to avoid off-target cells and tissues. Indeed, the company's siRNA therapeutics are specifically engineered to perfectly match their target mRNAs. The company has six drugs in development; two have reached phase 2 clinical trials (Table 1), while the remaining four drugs are in the preclinical stage. These drugs in the clinical stages are introduced below.

Zerlasiran (SLN360)[51] is a GalNAc-siRNA combination designed to target lipoprotein(a) (LPA) to treat cardiovascular disorders linked to LPA by subcutaneous administration. Two clinical trials have been performed. The phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT04606602)[52] was completed in August 2023 and demonstrated that this drug reduces LPA by 98% after a single dose in 32 healthy volunteers. Moreover, the effect lasts for more than 5 months, with good tolerability and no detected clinically significant safety issues. The phase 2 trial (this study was registered at ClinicalTrials.gov: NCT05537571) aims to investigate the efficacy, safety, and tolerability of SLN360 in participants with high LPA levels who are at high risk of ASCVD. The trial started in January 2023, and the results have not yet been released.

SLN-124 is a GalNAc-siRNA combination designed to specifically target the transmembrane protease serine 6 (TMPRSS6) for the treatment of β-thalassemia, myelodysplastic syndromes (MDSs), and polycythemia vera. A total of four clinical trials have been carried out—two have been completed, one has been interrupted, and one is in progress. The safety and tolerability of SLN124 were assessed in a phase 1 trial (this study was registered at ClinicalTrials.gov: NCT04559971)[53] on 24 healthy volunteers, with doses of 1.0, 3.0, and 4.5 mg/kg. The trial also evaluated pharmacokinetic characteristics and pharmacodynamic biomarkers of iron metabolism to determine the decrease in iron. All SLN124 dosages resulted in a substantial decrease in transferrin saturation (TSAT). The achieved absolute level of TSAT (10%–16%) is lower than the level at which tissue iron use becomes limited (<20%) and is equal to or below the level required to support normal erythrocyte production for health (<16%).

Another phase 1 trial (this study was registered at ClinicalTrials.gov: NCT04718844) involved adult subjects with α-/β-thalassemia and MDSs at low and very low risk. The objective was to assess the effectiveness and safety of SLN124. No significant negative effects were observed after receiving a single dose, such as SAEs and treatment-emergent adverse events (TEAEs) associated with SLN124 or TEAEs that lead to treatment interruption. No toxicity was observed with the maximum allowable dose or any instance of liver injury caused by the medicine. A clinical trial (this study was registered at ClinicalTrials.gov: NCT05499013) is under way to investigate the effect of increasing dosage in individuals with polycythemia vera. The trial is designed as a randomized, double-blind phase 1/2 study.

Other companies

In addition to the aforementioned companies specializing in the creation of siRNA medications, several other companies are involved in the development of siRNA drugs. An overview of these corporations and their siRNA medications are listed below.

Phio Pharmaceuticals specializes in RNAi and performs research on its use in the field of immuno-oncology. This company possesses a distinctive self-delivering siRNA technology known as INTASYL, which is primarily directed to immuno-oncology.

PH-762 (Table 1) is an RNAi molecule that blocks the activity of programmed cell death protein 1[54] used in cell-based cancer immunotherapy, specifically in a treatment called adoptive cell therapy. It uses RXi Pharmaceuticals' self-delivering RNAi technology to treat melanoma and other solid cancers. The medication has been in a phase 1 clinical trial (this study was registered at ClinicalTrials.gov: NCT06014086) since November 2023 to assess its efficacy in treating cutaneous squamous cell carcinoma, melanoma, or Merkel cell carcinoma by a dose-increasing manner.

PH-894 (Table 1) is an RNAi molecule that enters tumor cells and suppresses the activity of the bromodomain-containing protein 4 (BRD4).[55] This medication is specifically designed to treat advanced stage IV melanoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, Merkel cell carcinoma, and other types of solid tumors. It is scheduled to undergo a phase 1 trial (TrialTroveID-467142) to assess its pharmacodynamics in patients with solid tumors.

Amgen's olpasiran (Table 1),[56] previously known as AMG 890, is a therapeutic medication that specifically targets and reduces the levels of LPA and is undergoing research and development for its potential use in treating ASCVD. It is currently in phase 3 trials, with the completion of five linked trial programs. A phase 1 trial (this study was registered at ClinicalTrials.gov: NCT03626662)[57] assessed its safety, tolerability, pharmacokinetics, and pharmacodynamics in individuals with high levels of LPA in their blood plasma, with the main objective being the assessment of the safety and tolerability of the treatment, as well as any changes in LPA concentration and the pharmacokinetic properties as secondary outcomes. Participants tolerated well the single doses of olpasiran, resulting in a significant reduction in LPA values by 71%–97%. The effect of the administration of 9 mg or higher doses lasts for several months. The serum concentration of the drug is directly correlated with the dose. These data confirm that liver-targeted siRNA successfully lowers high plasma LPA levels. The drug was already subjected to phase 1 study in China (this study was registered at ClinicalTrials.gov: NCT04987320). A phase 2 trial (this study was registered at ClinicalTrials.gov: NCT04270760)[58] performed in December 2021 reported that patients receiving a dose $\geq$75 mg Q12W experienced a reduction of almost 95% in LPA levels at week 36. The results of the non-treatment extension period showed that patients who had previously taken 75 mg or more of the medication experienced an approximate 40%–50% reduction in placebo-adjusted LPA percentage nearly 1 year after their last administration. No additional safety concerns were observed during the extended time of the treatment. A clinical trial with the registration number NCT05581303 is under way to assess the impact of olpasiran on ASCVD and increased LPA. The study is being performed at multiple centers and follows a double-blind, randomized, placebo-controlled design and started in December 2022.

Arbutus Biopharma's imdusiran (AB-729)[59] (Table 1) is a GalNAc RNAi therapy that specifically targets HBsAg or other HBV targets. This medication uses Arbutus Biopharma's unique GalNAc conjugate technology. The clinical trials arrived at phase 2. The results of the initial phase 1 trial (TrialTroveID-334421) showed that the average levels of HBsAg remained lower than the initial levels after 48 weeks of follow-up. An evident and consistent reduction in HBsAg levels was observed during and after treatment with imdusiran. Additionally, this drug was generally safe and well tolerated after 48 weeks of repeated administration, with no adverse events related to the treatment or any need for discontinuation due to adverse events. The medicine is undergoing evaluation in phase 2a research (TrialTroveID-426794), which is randomized, blinded, and performed at many centers, with the aim of analyzing the safety and reactogenicity of AB-729 in conjunction with VTP-300 in people with chronic hepatitis B who underwent virologic suppression.

SUMMARY AND OUTLOOK

siRNA drugs are gradually being introduced into the market, offering advantages such as high efficiency and low dosing frequency. These drugs are becoming increasingly mature through a series of innovations in the optimization of delivery and advancements in chemical modifications. Many medications on the market are designed to affect the liver, while some drugs are being tested in clinical trials that target tissues outside the liver. The transition from the preclinical phase to the market for a successful siRNA medicine consists of six essential stages: target screening, drug design and synthesis, screening of the delivery strategy and chemical modifications, proof of concept (POC) in animals, clinical POC, and finally, regulatory approval.[60]

Most screening targets are currently disease targets by analyzing upregulated biomarkers in disease tissues or animal models. However, the therapeutic outcome of inhibiting or knocking down a disease

target is frequently ineffective, probably because of pathological compensations or other factors. The upstream signaling analysis of the interactions with the target or multi-target co-inhibition to achieve synergy is performed to reach the desired effect. Patient biopsies are the best samples for analysis, and the best results are obtained by analyzing many complex samples using genetics, multi-omics, and artificial intelligence.[61] In addition to being silent inside the target tissue, the target should also be secure enough to be silent outside of the target tissue. Target selection must also include the influence of the proliferation rate of the cell type containing the target gene.[62] Finally, target selection must take into account the impact of the turnover mRNA rates[63] that, when high, counteract the silencing mediated by RNAi.

Genetic data such as mRNA expression patterns, SNPs, sequence specificity, homology, and functionality must be analyzed to design the appropriate drug. Computer technology is used to anticipate the cross-species targeting, functionality, and specificity of siRNAs to increase the probability that the use of a specific siRNA will be successful. The correlation between siRNA effect *in vitro*, *in vivo*, and in clinical settings is often low, thus requiring innovation to improve the predictive accuracy. Machine learning is used to advance this development.[64] Drug design involves the comparison of mRNA transcripts from different species using databases and analyzing the homology[65] of target sequences in humans and experimental animals. Regions with identical sequences are often preferred targets, and this process accelerates the overall pace of drug development.

The method of drug synthesis is constantly evolving. In the past, siRNA synthesis through phosphoramidite chemistry was complex,[66] involving a four-step cycle of deprotection, coupling, capping, and oxidation.[67] However, this method no longer meets the demand of large-scale production of siRNA drugs due to high risks, significant consumption of raw material,[68] minimal product yield,[69] and poor economic profitability. These problems have become barriers in the development of siRNA drugs. Enzymatic synthesis of oligonucleotides has improved these problems[70] by enabling high-yield and high-purity siRNA production. This approach optimizes the traditional four-step chemical synthesis using a simplified two-step extension and deblocking process,[71] reducing the use of reagents. Additionally, the aqueous-based process avoids the extensive consumption of organic solvents and harmful by-products,[72] thus enhancing atom economy.

Two more important aspects of a functional design are effective delivery and chemical alteration. The most common delivery mechanism currently used is suffix-mediated delivery; extrahepatic delivery through aptamers,[73] lipids,[74] antibodies,[75] modified proteins,[76] peptides,[77] and other targeting affixes is still being developed. GalNAc-mediated intrahepatic delivery is also efficient and long-lasting. siRNAs have significant effects on the eyes, skin,[78] lungs, and central nervous system,[79] in addition to that on the liver. The effects of these drugs on other tissues, such as heart and kidney are being explored. The four most common chemical modifications at the moment are 2′-OMe,[80] 2′-F,[81] PS,[82] and 5′-(E)-vinylphosphonate[83] combinations. These modifications may be applied to most sequences and have different benefits, including improved siRNA stability, decreased toxicity, and extended drug persistence.

Animal POC is a POC that *in vivo* drugs have a statistically significant silent effect on a target at a safe and tolerable dose in humans. After siRNAs are designed, they should first be evaluated in disease models. For instance, the relevant species are monkeys and rats. A variety of tests are performed, including *in vitro* and *in vivo* pharmacodynamic studies; safety pharmacology studies; absorption, distribution, metabolism, and excretion studies; repeated dose toxicity studies in rats; repeated dose toxicity studies in cynomolgus monkeys; genetic toxicity tests; carcinogenicity studies; and reproductive and developmental toxicity studies.

Clinical POC is categorized into phase 1, 2, and 3 clinical trials. Phase 1 clinical trials are designed to test the safety, maximum tolerated dose, pharmacokinetics, and pharmacodynamics of a drug, and drug-drug interactions and are usually performed in a small number of healthy volunteers and patients. Phase 2 clinical trials are larger than phase 1 and are carried out in a patient population to evaluate safety, pharmacokinetics, and pharmacodynamics. They also explore the planning of phase 3 trials to determine the optimal dose, optimal frequency, and dosing endpoints. Phase 3 trials use a larger and more diverse target population to evaluate the drug and are designed to determine the efficacy and the incidence of adverse effects. Only after the completion of these phases is an siRNA drug marketed with a regulatory approval.

In conclusion, siRNA drugs are crucial at every stage from research to market launch. Each stage has driven the rapid development of siRNA drugs, from the continuous optimization of target screening, drug design, and synthesis methods to the technological innovation of delivery strategies and chemical modifications. siRNA treatments show considerable promise in various disease areas, including genetic disorders, metabolic diseases, cardiovascular diseases, neurological ailments, cancer, and immune system disorders. A few medications are already approved and have demonstrated encouraging therapeutic results, while the majority of the drugs are still in research and development. In the future, siRNA drugs are expected to occupy an important position in the global pharmaceutical market with the completion of more research and the launch of new products.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (no. 82403686), the Natural Science Foundation of Jiangsu Province (no. BK20241620), the China Postdoctoral Science Foundation (no. 2024M751160), and the Jiangsu Funding Program for Excellent Postdoctoral Talent (no. 2024ZB069). We would like to thank MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization: B.X. and R.W. Investigation: R.W., Y.P., C.G., T.G., and S.W. Funding acquisition: R.W. Project administration: R.W., Y.Q.C., and W.Z. Supervision: R.W. and

Y.Q.C. Writing – original draft: B.X. Writing – review & editing: R.W., J.Z., S.W., and C.L. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*, 806–811.
2. Spiegelman, W.G., Reichardt, L.F., Yaniv, M., Heinemann, S.F., Kaiser, A.D., and Eisen, H. (1972). Bidirectional transcription and the regulation of Phage lambda repressor synthesis. Proc. Natl. Acad. Sci. USA *69*, 3156–3160.
3. Lindbo, J.A., and Dougherty, W.G. (1992). Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology *189*, 725–733.
4. Waterhouse, P.M., Graham, M.W., and Wang, M.B. (1998). Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc. Natl. Acad. Sci. USA *95*, 13959–13964.
5. Setten, R.L., Rossi, J.J., and Han, S.P. (2019). The current state and future directions of RNAi-based therapeutics. Nat. Rev. Drug Discov. *18*, 421–446.
6. Liang, X., Zhang, J., Ou, H., Chen, J., Mitragotri, S., and Chen, M. (2020). Skin Delivery of siRNA Using Sponge Spicules in Combination with Cationic Flexible Liposomes. Mol. Ther. Nucleic Acids *20*, 639–648.
7. Cao, Y., Zhang, S., Ma, M., and Zhang, Y. (2022). Fluorinated PEG-PEI Coated Magnetic Nanoparticles for siRNA Delivery and CXCR4 Knockdown. Nanomaterials *12*, 1692.
8. Banks, T.M., Wang, T., Fitzgibbon, Q.P., Smith, G.G., and Ventura, T. (2020). Double-Stranded RNA Binding Proteins in Serum Contribute to Systemic RNAi Across Phyla-Towards Finding the Missing Link in Achelata. Int. J. Mol. Sci. *21*, 6967.
9. Alshaer, W., Zureigat, H., Al Karaki, A., Al-Kadash, A., Gharaibeh, L., Hatmal, M.M., Aljabali, A.A.A., and Awidi, A. (2021). siRNA: Mechanism of action, challenges, and therapeutic approaches. Eur. J. Pharmacol. *905*, 174178.
10. McMahon, K.M., Plebanek, M.P., and Thaxton, C.S. (2016). Properties of Native High-Density Lipoproteins Inspire Synthesis of Actively Targeted In Vivo siRNA Delivery Vehicles. Adv. Funct. Mater. *26*, 7824–7835.
11. Liao, L., Cen, B., Li, G., Wei, Y., Wang, Z., Huang, W., He, S., Yuan, Y., and Ji, A. (2021). A bivalent cyclic RGD-siRNA conjugate enhances the antitumor effect of apatinib via co-inhibiting VEGFR2 in non-small cell lung cancer xenografts. Drug Deliv. *28*, 1432–1442.
12. Luo, Y., Liu, L., He, Z., Zhang, S., Huo, P., Wang, Z., Jiaxin, Q., Zhao, L., Wu, Y., Zhang, D., et al. (2022). TREAT: Therapeutic RNAs exploration inspired by artificial intelligence technology. Comput. Struct. Biotechnol. J. *20*, 5680–5689.
13. Hoy, S.M. (2018). Patisiran: First Global Approval. Drugs *78*, 1625–1631.
14. Scott, L.J. (2020). Givosiran: First Approval. Drugs *80*, 335–339.
15. Scott, L.J., and Keam, S.J. (2021). Lumasiran: First Approval. Drugs *81*, 277–282.
16. Keam, S.J. (2022). Vutrisiran: First Approval. Drugs *82*, 1419–1425.
17. Lamb, Y.N. (2021). Inclisiran: First Approval. Drugs *81*, 389–395.
18. Syed, Y.Y. (2023). Nedosiran: First Approval. Drugs *83*, 1729–1733.
19. Pasi, K.J., Rangarajan, S., Georgiev, P., Mant, T., Creagh, M.D., Lissitchkov, T., Bevan, D., Austin, S., Hay, C.R., Hegemann, I., et al. (2017). Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy. N. Engl. J. Med. *377*, 819–828.
20. Badri, P., Jiang, X., Borodovsky, A., Najafian, N., Kim, J., Clausen, V.A., Goel, V., Habtemariam, B., and Robbie, G.J. (2021). Pharmacokinetic and Pharmacodynamic Properties of Cemdisiran, an RNAi Therapeutic Targeting Complement Component 5, in Healthy Subjects and Patients with Paroxysmal Nocturnal Hemoglobinuria. Clin. Pharmacokinet. *60*, 365–378.
21. Remih, K., Amzou, S., and Strnad, P. (2021). Alpha1-antitrypsin deficiency: New therapies on the horizon. Curr. Opin. Pharmacol. *59*, 149–156.
22. Gane, E., Lim, Y.S., Kim, J.B., Jadhav, V., Shen, L., Bakardjiev, A.I., Huang, S.A., Cathcart, A.L., Lempp, F.A., Janas, M.M., et al. (2023). Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: Results from randomized clinical trials. J. Hepatol. *79*, 924–932.
23. Desai, A.S., Webb, D.J., Taubel, J., Casey, S., Cheng, Y., Robbie, G.J., Foster, D., Huang, S.A., Rhyee, S., Sweetser, M.T., and Bakris, G.L. (2023). Zilebesiran, an RNA Interference Therapeutic Agent for Hypertension. N. Engl. J. Med. *389*, 228–238.
24. Bakris, G.L., Saxena, M., Gupta, A., Chalhoub, F., Lee, J., Stiglitz, D., Makarova, N., Goyal, N., Guo, W., Zappe, D., et al. (2024). RNA Interference With Zilebesiran for Mild to Moderate Hypertension: The KARDIA-1 Randomized Clinical Trial. JAMA *331*, 740–749.
25. Zhang, H.B., Su, W., Xu, H., Zhang, X.Y., and Guan, Y.F. (2021). HSD17B13: A Potential Therapeutic Target for NAFLD. Front. Mol. Biosci. *8*, 824776.
26. O'Brien, R.J., and Wong, P.C. (2011). Amyloid precursor protein processing and Alzheimer's disease. Annu. Rev. Neurosci. *34*, 185–204.
27. Teixeira, C., Martins, H.S., and Saraiva, M.J. (2023). Cellular environment of TTR deposits in an animal model of ATTR-Cardiomyopathy. Front. Mol. Biosci. *10*, 1144049.
28. Park, J., Zhao, Y., Zhang, F., Zhang, S., Kwong, A.C., Zhang, Y., Hoffmann, H.H., Bushweller, L., Wu, X., Ashbrook, A.W., et al. (2023). IL-6/STAT3 axis dictates the PNPLA3-mediated susceptibility to non-alcoholic fatty liver disease. J. Hepatol. *78*, 45–56.
29. Gutierrez, J.A., Liu, W., Perez, S., Xing, G., Sonnenberg, G., Kou, K., Blatnik, M., Allen, R., Weng, Y., Vera, N.B., et al. (2021). Pharmacologic inhibition of ketohexokinase prevents fructose-induced metabolic dysfunction. Mol. Metabol. *48*, 101196.
30. Rim, E.Y., Clevers, H., and Nusse, R. (2022). The Wnt Pathway: From Signaling Mechanisms to Synthetic Modulators. Annu. Rev. Biochem. *91*, 571–598.
31. Strnad, P., Mandorfer, M., Choudhury, G., Griffiths, W., Trautwein, C., Loomba, R., Schluep, T., Chang, T., Yi, M., Given, B.D., et al. (2022). Fazirsiran for Liver Disease Associated with Alpha(1)-Antitrypsin Deficiency. N. Engl. J. Med. *387*, 514–524.
32. Ballantyne, C.M., Vasas, S., Azizad, M., Clifton, P., Rosenson, R.S., Chang, T., Melquist, S., Zhou, R., Mushin, M., Leeper, N.J., et al. (2024). Plozasiran, an RNA Interference Agent Targeting APOC3, for Mixed Hyperlipidemia. N. Engl. J. Med. *391*, 899–912.
33. Gaudet, D., Pall, D., Watts, G.F., Nicholls, S.J., Rosenson, R.S., Modesto, K., San Martin, J., Hellawell, J., and Ballantyne, C.M. (2024). Plozasiran (ARO-APOC3) for Severe Hypertriglyceridemia: The SHASTA-2 Randomized Clinical Trial. JAMA Cardiol *9*, 620–630.
34. Watts, G.F., Schwabe, C., Scott, R., Gladding, P.A., Sullivan, D., Baker, J., Clifton, P., Hamilton, J., Given, B., Melquist, S., et al. (2023). RNA interference targeting ANGPTL3 for triglyceride and cholesterol lowering: phase 1 basket trial cohorts. Nat. Med. *29*, 2216–2223.
35. Mak, L.Y., Gane, E., Schwabe, C., Yoon, K.T., Heo, J., Scott, R., Lee, J.H., Lee, J.I., Kweon, Y.O., Weltman, M., et al. (2023). A phase I/II study of ARO-HSD, an RNA interference therapeutic, for the treatment of non-alcoholic steatohepatitis. J. Hepatol. *78*, 684–692.
36. Yuen, M.F., Locarnini, S., Lim, T.H., Strasser, S.I., Sievert, W., Cheng, W., Thompson, A.J., Given, B.D., Schluep, T., Hamilton, J., et al. (2022). Combination treatments including the small-interfering RNA JNJ-3989 induce rapid and sometimes prolonged viral responses in patients with CHB. J. Hepatol. *77*, 1287–1298.
37. Zhang, G., Jiang, W., He, F., Fu, J., Xu, X., Luo, X., and Cao, Z. (2024). LDL-C and TC mediate the risk of PNPLA3 inhibition on cardiovascular diseases. J. Clin. Endocrinol. Metab. *18*, 264.
38. Dreismann, A.K., Hallam, T.M., Tam, L.C., Nguyen, C.V., Hughes, J.P., Ellis, S., and Harris, C.L. (2023). Gene targeting as a therapeutic avenue in diseases mediated by the complement alternative pathway. Immunol. Rev. *313*, 402–419.
39. Akhlaghpour, M., Haritunians, T., More, S.K., Thomas, L.S., Stamps, D.T., Dube, S., Li, D., Yang, S., Landers, C.J., Mengesha, E., et al. (2023). Genetic coding variant in complement factor B (CFB) is associated with increased risk for perianal Crohn's disease and leads to impaired CFB cleavage and phagocytosis. Gut *72*, 2068–2080.

40. Okuda, K., Chen, G., Subramani, D.B., Wolf, M., Gilmore, R.C., Kato, T., Radicioni, G., Kesimer, M., Chua, M., Dang, H., et al. (2019). Localization of Secretory Mucins MUC5AC and MUC5B in Normal/Healthy Human Airways. Am. J. Respir. Crit. Care Med. *199*, 715–727.

41. Koerich, S., Parreira, G.M., de Almeida, D.L., Vieira, R.P., and de Oliveira, A.C.P. (2023). Receptors for Advanced Glycation End Products (RAGE): Promising Targets Aiming at the Treatment of Neurodegenerative Conditions. Curr. Neuropharmacol. *21*, 219–234.

42. Hirohama, D., Abedini, A., Moon, S., Surapaneni, A., Dillon, S.T., Vassalotti, A., Liu, H., Doke, T., Martinez, V., Md Dom, Z., et al. (2023). Unbiased Human Kidney Tissue Proteomics Identifies Matrix Metalloproteinase 7 as a Kidney Disease Biomarker. J. Am. Soc. Nephrol. *34*, 1279–1291.

43. Duranti, E., and Villa, C. (2023). Influence of DUX4 Expression in Facioscapulohumeral Muscular Dystrophy and Possible Treatments. Int. J. Mol. Sci. *24*, 9503.

44. Pantic, B., Trevisan, E., Citta, A., Rigobello, M.P., Marin, O., Bernardi, P., Salvatori, S., and Rasola, A. (2013). Myotonic dystrophy protein kinase (DMPK) prevents ROS-induced cell death by assembling a hexokinase II-Src complex on the mitochondrial surface. Cell Death Dis. *4*, e858.

45. Atiya, A., Muhsinah, A.B., Alrouji, M., Alhumaydhi, F.A., Al Abdulmonem, W., Aljasir, M.A., Sharaf, S.E., Furkan, M., Khan, R.H., Shahwan, M., and Shamsi, A. (2023). Unveiling promising inhibitors of superoxide dismutase 1 (SOD1) for therapeutic interventions. Int. J. Biol. Macromol. *253*, 126684.

46. Bortolotti, M., Polito, L., Battelli, M.G., and Bolognesi, A. (2021). Xanthine oxidoreductase: One enzyme for multiple physiological tasks. Redox Biol. *41*, 101882.

47. Wang, J., Chen, G., Liu, N., Han, X., Zhao, F., Zhang, L., and Chen, P. (2022). Strategies for improving the safety and RNAi efficacy of noncovalent peptide/siRNA nanocomplexes. Adv. Colloid Interface Sci. *302*, 102638.

48. Kuźbicki, Ł., and Brożyna, A.A. (2021). Expression of Cyclooxygenase-2 in Human Epithelial Skin Lesions: A Systematic Review of Immunohistochemical Studies. Appl. Immunohistochem. Mol. Morphol. *29*, 163–174.

49. Huang, R., Yu, J., Zhang, B., Li, X., Liu, H., and Wang, Y. (2025). Emerging COX-2 inhibitors-based nanotherapeutics for cancer diagnosis and treatment. Biomaterials *315*, 122954.

50. De Caterina, R., Prisco, D., and Eikelboom, J.W. (2023). Factor XI inhibitors: cardiovascular perspectives. Eur. Heart J. *44*, 280–292.

51. Nissen, S.E., Wolski, K., Watts, G.F., Koren, M.J., Fok, H., Nicholls, S.J., Rider, D.A., Cho, L., Romano, S., Melgaard, C., and Rambaran, C. (2024). Single Ascending and Multiple-Dose Trial of Zerlasiran, a Short Interfering RNA Targeting Lipoprotein(a): A Randomized Clinical Trial. JAMA *331*, 1534–1543.

52. Nissen, S.E., Wolski, K., Balog, C., Swerdlow, D.I., Scrimgeour, A.C., Rambaran, C., Wilson, R.J., Boyce, M., Ray, K.K., Cho, L., et al. (2022). Single Ascending Dose Study of a Short Interfering RNA Targeting Lipoprotein(a) Production in Individuals With Elevated Plasma Lipoprotein(a) Levels. JAMA *327*, 1679–1687.

53. Porter, J.B., Scrimgeour, A., Martinez, A., James, L., Aleku, M., Wilson, R., Muckenthaler, M., Boyce, M., Wilkes, D., Schaeper, U., and Campion, G.V. (2023). SLN124, a GalNAc conjugated 19-mer siRNA targeting tmprss6, reduces plasma iron and increases hepcidin levels of healthy volunteers. Am. J. Hematol. *98*, 1425–1435.

54. Han, Y., Liu, D., and Li, L. (2020). PD-1/PD-L1 pathway: current researches in cancer. Am. J. Cancer Res. *10*, 727–742.

55. Yang, M., Liu, K., Chen, P., Zhu, H., Wang, J., and Huang, J. (2022). Bromodomain-containing protein 4 (BRD4) as an epigenetic regulator of fatty acid metabolism genes and ferroptosis. Cell Death Dis. *13*, 912.

56. Duarte Lau, F., and Giugliano, R.P. (2022). Lipoprotein(a) and its Significance in Cardiovascular Disease: A Review. JAMA Cardiol. *7*, 760–769.

57. Koren, M.J., Moriarty, P.M., Baum, S.J., Neutel, J., Hernandez-Illas, M., Weintraub, H.S., Florio, M., Kassahun, H., Melquist, S., Varrieur, T., et al. (2022). Preclinical development and phase 1 trial of a novel siRNA targeting lipoprotein(a). Nat. Med. *28*, 96–103.

58. O'Donoghue, M.L., Rosenson, R.S., Gencer, B., López, J.A.G., Lepor, N.E., Baum, S.J., Stout, E., Gaudet, D., Knusel, B., Kuder, J.F., et al. (2022). Small Interfering RNA to Reduce Lipoprotein(a) in Cardiovascular Disease. N. Engl. J. Med. *387*, 1855–1864.

59. Anderson, M., Stec, M., Thi, E.P., Picchio, G., Mbanya, D., and Cloherty, G. (2023). Measuring hepatitis B pgRNA stability using an updated automated HBV pgRNA assay with increased sensitivity. Hepatol. Commun. *7*, e0099.

60. Tang, Q., and Khvorova, A. (2024). RNAi-based drug design: considerations and future directions. Nat. Rev. Drug Discov. *23*, 341–364.

61. Trajanoska, K., Bhérer, C., Taliun, D., Zhou, S., Richards, J.B., and Mooser, V. (2023). From target discovery to clinical drug development with human genetics. Nature *620*, 737–745.

62. Judge, A.D., Robbins, M., Tavakoli, I., Levi, J., Hu, L., Fronda, A., Ambegia, E., McClintock, K., and MacLachlan, I. (2009). Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. J. Clin. Invest. *119*, 661–673.

63. Larsson, E., Sander, C., and Marks, D. (2010). mRNA turnover rate limits siRNA and microRNA efficacy. Mol. Syst. Biol. *6*, 433.

64. Monopoli, K.R., Korkin, D., and Khvorova, A. (2023). Asymmetric trichotomous partitioning overcomes dataset limitations in building machine learning models for predicting siRNA efficacy. Mol. Ther. Nucleic Acids *33*, 93–109.

65. Tang, Q., Gross, K.Y., Fakih, H.H., Jackson, S.O., Zain U I Abideen, M., Monopoli, K.R., Blanchard, C., Bouix-Peter, C., Portal, T., Harris, J.E., et al. (2024). Multispecies-targeting siRNAs for the modulation of JAK1 in the skin. Mol. Ther. Nucleic Acids *35*, 102117.

66. Beaucage, S.L. (2008). Solid-phase synthesis of siRNA oligonucleotides. Curr. Opin. Drug Discov. Dev *11*, 203–216.

67. Kosuri, S., and Church, G.M. (2014). Large-scale de novo DNA synthesis: technologies and applications. Nat. Methods *11*, 499–507.

68. Moody, E.R., Obexer, R., Nickl, F., Spiess, R., and Lovelock, S.L. (2023). An enzyme cascade enables production of therapeutic oligonucleotides in a single operation. Science *380*, 1150–1154.

69. Tedebark, U., Scozzari, A., Werbitzky, O., Capaldi, D., and Holmberg, L. (2011). Industrial-scale manufacturing of a possible oligonucleotide cargo CPP-based drug. Methods Mol. Biol. *683*, 505–524.

70. Jensen, M.A., and Davis, R.W. (2018). Template-Independent Enzymatic Oligonucleotide Synthesis (TiEOS): Its History, Prospects, and Challenges. Biochemistry *57*, 1821–1832.

71. Wiegand, D.J., Rittichier, J., Meyer, E., Lee, H., Conway, N.J., Ahlstedt, D., Yurtsever, Z., Rainone, D., Kuru, E., and Church, G.M. (2024). Template-independent enzymatic synthesis of RNA oligonucleotides. Nat. Biotechnol. *12*, 1–15.

72. Andrews, B.I., Antia, F.D., Brueggemeier, S.B., Diorazio, L.J., Koenig, S.G., Kopach, M.E., Lee, H., Olbrich, M., and Watson, A.L. (2021). Sustainability Challenges and Opportunities in Oligonucleotide Manufacturing. J. Org. Chem. *86*, 49–61.

73. McNamara, J.O., 2nd, Andrechek, E.R., Wang, Y., Viles, K.D., Rempel, R.E., Gilboa, E., Sullenger, B.A., and Giangrande, P.H. (2006). Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat. Biotechnol. *24*, 1005–1015.

74. Biscans, A., Coles, A., Haraszti, R., Echeverria, D., Hassler, M., Osborn, M., and Khvorova, A. (2019). Diverse lipid conjugates for functional extra-hepatic siRNA delivery in vivo. Nucleic Acids Res. *47*, 1082–1096.

75. Nanna, A.R., Kel'in, A.V., Theile, C., Pierson, J.M., Voo, Z.X., Garg, A., Nair, J.K., Maier, M.A., Fitzgerald, K., and Rader, C. (2020). Generation and validation of structurally defined antibody-siRNA conjugates. Nucleic Acids Res. *48*, 5281–5293.

76. Klein, D., Goldberg, S., Theile, C.S., Dambra, R., Haskell, K., Kuhar, E., Lin, T., Parmar, R., Manoharan, M., Richter, M., et al. (2021). Centyrin ligands for extrahepatic delivery of siRNA. Mol. Ther. *29*, 2053–2066.

77. Ämmälä, C., Drury, W.J., 3rd, Knerr, L., Ahlstedt, I., Stillemark-Billton, P., Wennberg-Huldt, C., Andersson, E.M., Valeur, E., Jansson-Löfmark, R., Janzén, D., et al. (2018). Targeted delivery of antisense oligonucleotides to pancreatic β-cells. Sci. Adv. *4*, eaat3386.

78. Tang, Q., Fakih, H.H., Zain Ui Abideen, M., Hildebrand, S.R., Afshari, K., Gross, K.Y., Sousa, J., Maebius, A.S., Bartholdy, C., Søgaard, P.P., et al. (2023). Rational design of a JAK1-selective siRNA inhibitor for the modulation of autoimmunity in the skin. Nat. Commun. *14*, 7099.

79. Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E.M., Logroscino, G., Robberecht, W., Shaw, P.J., Simmons, Z., and van den Berg, L.H. (2017). Amyotrophic lateral sclerosis. Nat. Rev. Dis. Prim. *3*, 17071.

80. Schirle, N.T., Sheu-Gruttadauria, J., and MacRae, I.J. (2014). Structural basis for microRNA targeting. Science *346*, 608–613.

81. Manoharan, M. (1999). 2'-carbohydrate modifications in antisense oligonucleotide therapy: importance of conformation, configuration and conjugation. Biochim. Biophys. Acta *1489*, 117–130.

82. Foster, D.J., Brown, C.R., Shaikh, S., Trapp, C., Schlegel, M.K., Qian, K., Sehgal, A., Rajeev, K.G., Jadhav, V., Manoharan, M., et al. (2018). Advanced siRNA Designs Further Improve In Vivo Performance of GalNAc-siRNA Conjugates. Mol. Ther. *26*, 708–717.

83. Guenther, D.C., Mori, S., Matsuda, S., Gilbert, J.A., Willoughby, J.L.S., Hyde, S., Bisbe, A., Jiang, Y., Agarwal, S., Madaoui, M., et al. (2022). Role of a "Magic" Methyl: 2'-Deoxy-2'-α-F-2'-β-C-methyl Pyrimidine Nucleotides Modulate RNA Interference Activity through Synergy with 5'-Phosphate Mimics and Mitigation of Off-Target Effects. J. Am. Chem. Soc. *144*, 14517–14534.