

REVIEW ARTICLE

Gene Silencing Therapeutics in Cardiology: A Review Article

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Abstract

Therapeutics that inhibit enzymes, receptors, ion channels, and cotransporters have long been the mainstay of cardiovascular medicine. Now, oligonucleotide therapeutics offer a modern variation on this paradigm of protein inhibition. Rather than target a protein, however, small interfering ribonucleic acids and antisense oligonucleotides target the messenger RNA (mRNA) from which a protein is translated. Endogenous, cellular mechanisms enable the oligonucleotides to bind a selected sequence on a target mRNA, leading to its degradation. The catalytic nature of the process confers an advantage over the stoichiometric binding of traditional small molecule therapeutics to their respective protein targets. Advances in nucleic acid chemistry and delivery have enabled development of oligonucleotide therapeutics against a wide range of diseases, including hyperlipidemias and hereditary transthyretin-mediated amyloidosis with polyneuropathy. While most of these therapeutics were initially designed for rare diseases, recent clinical trials highlight the potential impact of oligonucleotides on more common forms of cardiovascular disease.

Introduction

Almost every drug targets a protein, the last step in the flow of genetic information from DNA through transcription and translation. However, as early as 1978, Zamecnik envisioned targeting the prior step and demonstrated that a 13-base oligonucleotide complementary to a sequence in the Rous sarcoma virus

Keywords

Cardiovascular Disease; Gene Silencing; RNA Interference, Oligonucleotides; Antisense; Amyloidosis.

inhibited translation of viral messenger RNA (mRNA) and the consequent oncogenic transformation of cells in culture. Furthermore, a chemical modification of the oligonucleotide prolonged its efficacy, presumably by slowing its degradation.^{1,2} This latter observation foreshadowed the chemistry that enables the remarkable duration of action of oligonucleotide therapeutics today.

Silencing the expression of a gene by targeting its mRNA via an oligonucleotide expands the therapeutic armamentarium, overcoming major hurdles such as the identification of “druggable” proteins and therapeutics that bind them. Furthermore, target proteins may not have a suitable drug binding site, or binding to off-target proteins may cause undesirable side effects. Oligonucleotides can circumvent these limitations and expedite the drug discovery process. With a target protein and the sequence of its coding gene, an antisense oligonucleotide (ASO) or a small interfering ribonucleic acid (siRNA) can be designed to bind to a complementary sequence on an mRNA with exquisite specificity. Different cellular mechanisms are co-opted to degrade the mRNA: RNase H1 (ASO) or RNA interference (RNAi) [RNAi]. The physiologic functions of RNase H1 and RNAi include antiviral defense,³ and the RNAi pathway is fundamental to controlling gene expression in diverse eukaryotic species.^{4,5}

ASOs are single-stranded oligonucleotides of 15-20 bases, designed to be complementary (antisense) to a target site on the mRNA transcript,^{6,7} to which they bind via Watson–Crick base pairing. The ASO chemical modification pattern determines whether the target mRNA is degraded or processed. The most widely used mechanism involves an endogenous nuclease, RNase H1, which recognizes chimeric DNA/RNA duplexes formed by the ASO and target mRNA (Figure 1A). Hence the ASO needs to contain at least 8-10 deoxyribonucleotide residues in the center, which can be flanked by other

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chemically modified residues. After recognizing the ASO/mRNA duplex, RNase H1 cleaves the mRNA, thereby reducing production of the target protein.

siRNAs are double-stranded RNAs, 19-25 base pairs in length, which utilize the highly conserved natural RNAi pathway. The siRNAs are designed to recruit and bind to an enzyme complex called RNA-induced silencing complex (RISC) to mediate degradation of their respective mRNA targets.^{4,5} During the loading process into RISC, the 2 strands of the siRNAs are separated (Figure 1B). The sense “passenger” strand is ejected, and the antisense “guide” strand remains loaded and “guides” the RISC to a complementary site on the target mRNA, where it binds through Watson–Crick base pairing.⁸ After cleaving the mRNA, the antisense strand remains bound to the enzyme; thus, in the catalytic action of siRNAs, a single molecule serves to cleave a large number of target mRNAs.

In this review, key technical advances in the evolution of oligonucleotide therapeutics, including ASOs and siRNAs approved or in late-stage clinical development for cardiovascular diseases, are discussed.

Methods

Based on standard review methods, a comprehensive literature search of the PubMed database was conducted

to find relevant published articles on gene silencing in cardiology. Search terms were gene+silencing+cardiac (2157 results), RNAi+cardiac (491), and antisense+oligonucleotide+cardiac (1131). Adding “clinical” and/or “phase” refined searches to include only therapeutics in, or previously in, clinical trials, resulting in 78 publications under consideration. Only English language articles were reviewed, no date limits were specified, and searches were undertaken up to July 20, 2020. Relevant publications identified from references of the retrieved articles were also included. Up-to-date information on clinical trials of siRNA and ASO therapeutics in cardiology was sought on the website <https://clinicaltrials.gov>.

Oligonucleotide therapeutics: evolution of a novel class of medicines

Oligonucleotide therapeutics, with ASOs and siRNAs representing the most prominent gene silencing technologies, have emerged as a new generation of precision medicines for an increasing number of diseases with high unmet medical needs, including cardiovascular diseases. However, unmodified oligonucleotides lack drug-like properties, and decades were spent optimizing their chemistry and developing advanced drug delivery systems that were required for these molecules to become drugs.

Oligonucleotide therapeutics are rapidly degraded and eliminated without the chemical modifications that

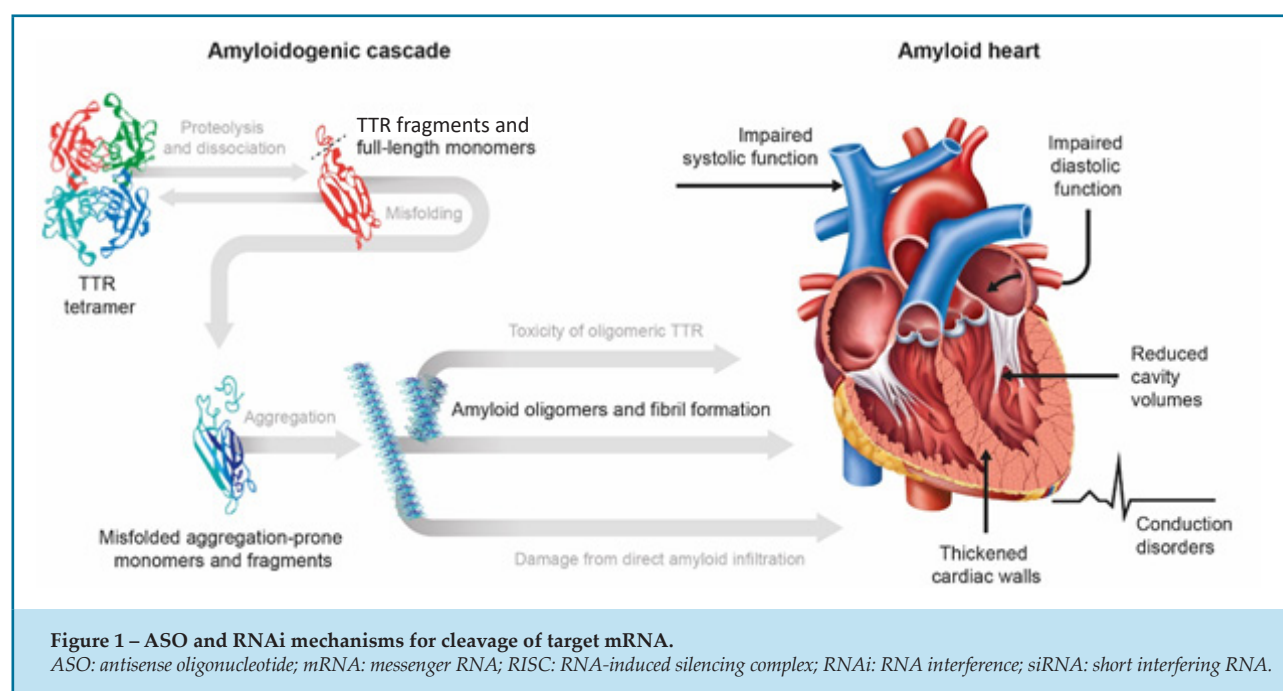


Figure 1 – ASO and RNAi mechanisms for cleavage of target mRNA.

ASO: antisense oligonucleotide; mRNA: messenger RNA; RISC: RNA-induced silencing complex; RNAi: RNA interference; siRNA: short interfering RNA.

assure stability and mediate delivery to the intended tissue. Ubiquitous nucleases hydrolyze the bond between phosphodiester groups in DNA/RNA and the sugar moiety of each base, thus degrading nucleic acids. A phosphorothioate modification (Figure 2A) protects the phosphodiester bond in ASOs and siRNAs against enzymatic hydrolysis⁹ and improves delivery of ASOs by increasing hydrophobicity and improving protein binding. Formation of ASO-protein complexes increases the ASO's half-life in circulation and reduces urinary excretion to facilitate distribution (predominantly) to liver, spleen, and kidney. While a phosphorothioate backbone confers advantages, it also elicits unwanted interactions with proteins, e.g., the platelet-specific glycoprotein receptor VI or complement, which may mediate thrombocytopenia or glomerulonephritis associated with ASOs.⁷

Chemical modifications of sugar moieties in an ASO or siRNA, such as 2'-O-methoxyethyl (2'-MOE), 2'-O-methyl (2'-O-Me), and 2'-fluoro (2'-F) (Figure 2A), are commonly incorporated to increase stability against nucleases, enhance avidity for the target mRNA, and mitigate any innate immune response.⁹ Of note, 2'-F and 2'-O-Me substitutions along the entire length of an siRNA (Figure 2A) can dramatically increase potency and duration of action.^{10,11}

Alongside advances in nucleic acid chemistry, highly effective drug delivery platforms were developed. Encapsulation of an siRNA in a lipid nanoparticle (LNP) represented a significant early advance in systemic delivery (Figure 2B). Demonstrating proof-of-concept, an LNP-siRNA formulation reduced hepatic apolipoprotein B (ApoB) mRNA and levels of ApoB and cholesterol in nonhuman primates.¹² Second-generation LNPs showed approximately 100-fold improved potency in mouse models.¹³ The improvement was translated to humans, leading to the first siRNA therapeutic, patisiran, for patients with hereditary transthyretin-mediated (hATTR) amyloidosis with polyneuropathy.^{14,15}

LNPs effectively deliver siRNAs to the liver, but manufacturing is complicated, and administration is intravenous. A simpler solution targets ASOs and siRNAs to cell surface receptors via a conjugated ligand that triggers endocytosis. A current strategy involves conjugation to an *N*-acetylgalactosamine (GalNAc) ligand (Figure 2B), which binds the asialoglycoprotein receptor (ASGPR). The ASGPR recognizes *N*-acetylgalactosamine residues on glycoproteins and is highly expressed on hepatocytes.^{16,17} High-affinity, multivalent GalNAc

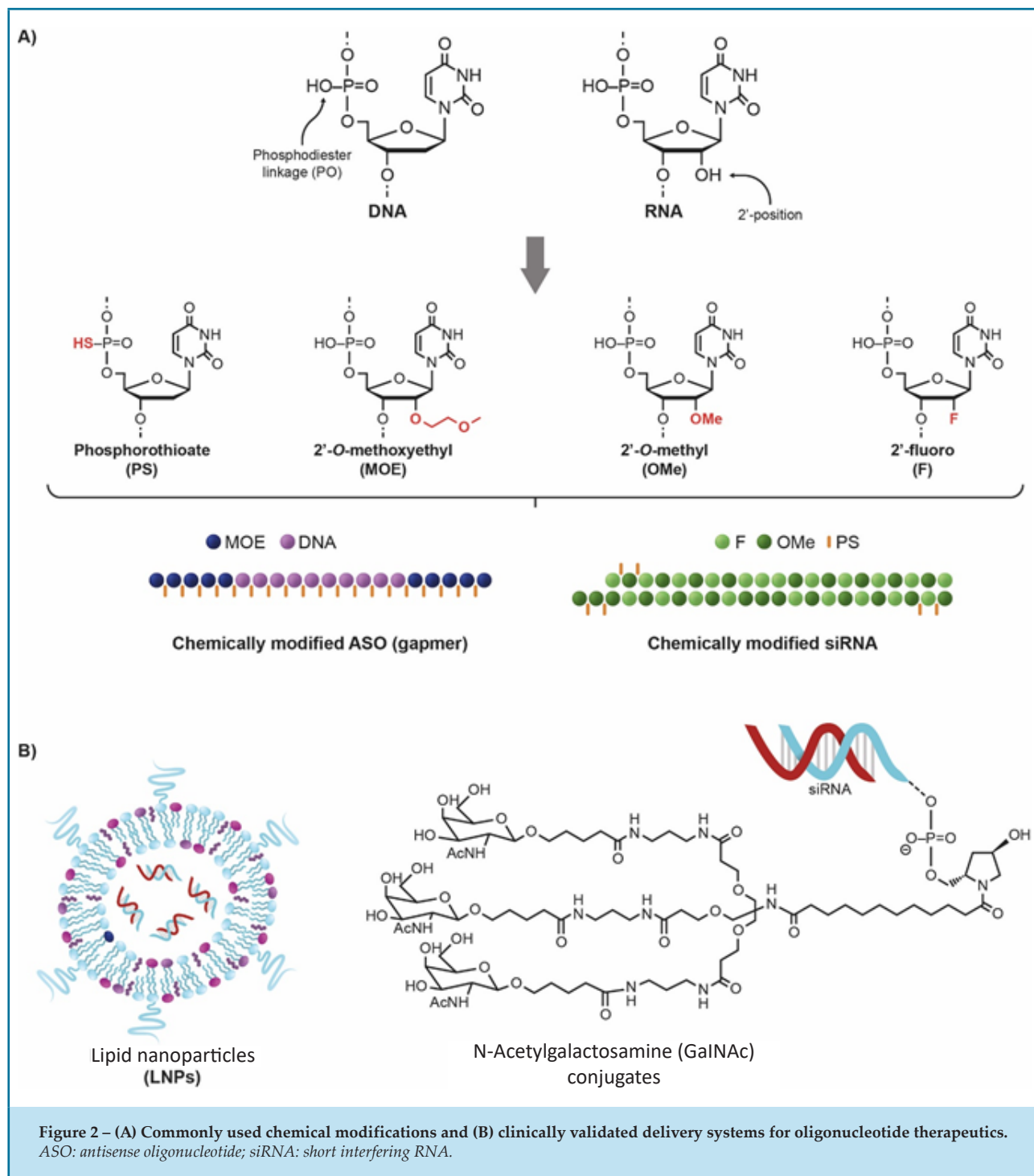
ligands suitable for subcutaneous administration have been developed for ASOs and siRNAs.^{11,18}

Chemically modified nucleic acids and GalNAc ligands have greatly expanded the potential of oligonucleotide therapeutics for diseases that can be treated by suppressing hepatic synthesis of a protein. The combination of stabilized siRNAs with a high-affinity GalNAc ligand, termed enhanced stabilization chemistry (ESC) conjugates, has enabled potent and durable gene silencing in the liver with infrequent administration at low doses.¹¹ The first GalNAc-siRNA conjugate approved was givosiran for the treatment of acute intermittent porphyria.¹⁹ The safety of the GalNAc-siRNA platform has been established in large clinical trials of inclisiran, discussed below.^{20,21} Several other GalNAc-ASO and -siRNA conjugates for cardiovascular indications are currently in clinical trials (Table 1).

Gene silencing in hyperlipidemias

Loss-of-function variants of proprotein convertase subtilisin/kexin type 9 (*PCSK9*), angiopoietin-like protein-3 (*ANGPTL3*), *APOC3* (ApoC-III), and *LPA* (ApoA) are associated with reduced low-density lipoprotein (LDL), cholesterol (LDL-C), or triglyceride levels and protection from coronary heart disease.^{22,23} Human genetics suggested that lowering circulating levels of these proteins could be used to treat hyperlipidemias and reduce cardiovascular risk. Large clinical trials of monoclonal antibodies against PCSK9 have since validated this therapeutic hypothesis.²⁴⁻²⁶ Oligonucleotide therapeutics targeting each of the genes above have been developed (Table 1). We discuss below those approved or having phase 3 clinical trial results.

Mipomersen, an ASO that suppresses ApoB synthesis, was the first oligonucleotide therapeutic approved for a cardiovascular indication. The rationale for its development in familial hypercholesterolemia was based in part on the phenotype of individuals with familial hypobetalipoproteinemia. Protein-truncating variants of *APOB* are associated with very low levels of LDL-C and ApoB,²⁷ and early clinical observations of humans who carried such *APOB* mutations suggested a reduced risk of atherosclerotic disease.²⁸ In initial clinical studies, mipomersen given weekly to patients with homozygous familial hypercholesterolemia reduced ApoB by 26.8% and LDL-C by 24.7% (from baseline 441 ± 139 mg/dL).²⁹ In subsequent phase 3 trials, mipomersen reduced LDL-C by 28-37% in patients with heterozygous familial



hypercholesterolemia or severe hypercholesterolemia, on maximally tolerated lipid-lowering therapeutics.³⁰⁻³² In all these trials, some patients developed elevations of hepatic transaminases and hepatic steatosis that resolved following discontinuation of mipomersen.³⁰⁻³² Familial hypobetalipoproteinemia is likewise associated with hepatic steatosis because ApoB is necessary for

export of triglycerides from the liver.³³ Mipomersen was withdrawn from the market.

Volanesorsen, an ASO that suppresses ApoC3 synthesis, is approved in the European Union (EU) for the treatment of familial chylomicronemia syndrome (FCS).³⁴ Characterized by hypertriglyceridemia and pancreatitis,

Table 1. Oligonucleotide therapies in mid- to late-stage development or approved for cardiovascular indications

Gene	Therapy	Disease indication	Clinical phase	Links
APOB	Mipomersen (ASO)	Familial hypercholesterolemia	Approved, but withdrawn from market	See text
APOC3	Volanesorsen (ASO)	Familial hyperchylomicronemia	Approved in some countries	See text
		Familial partial lipodystrophy	Phase 2	NCT02527343
	AKCEA-APOCIII-LRx (GalNAc-ASO)	Hypertriglyceridemia	Phase 3	NCT02300233
		Hypertriglyceridemia and cardiovascular disease	Phase 3	NCT03385239
ANGPTL3	AKCEA-ANGPTL3-LRx (GalNAc-ASO)	Familial chylomicronemia	Phase 2	NCT03360747
		Familial partial lipodystrophy	Phase 2	NCT03514420
		Hypertriglyceridemia, type 2 diabetes mellitus, and nonalcoholic fatty liver disease	Phase 2	NCT03371355
LPA (ApoA)	TQJ230 (GalNAc-ASO)	Cardiovascular disease with elevated Apolipoprotein A	Phase 3	NCT04023552
PCSK9	Inclisiran (GalNAc-siRNA)	Hypercholesterolemia	Phase 3	See text NCT03705234
	AKCEA-TTR-LRx (GalNAc-ASO)		Phase 3	See text NCT04136171
TTR	Patisiran (siRNA)	Transthyretin-mediated cardiac amyloidosis	Phase 3	See text NCT03997383
	Vutrisiran (GalNAc-siRNA)		Phase 3	See text NCT04153149

ANGPTL3: angiotensin-like protein-3; APO: apolipoprotein; ASO: antisense oligonucleotide; LPA: lipoprotein a ; GalNAc: N-Acetylgalactosamine; PCSK9: proprotein convertase subtilisin/kexin type 9; siRNA: short interfering RNA; TTR: transthyretin

FCS is caused by a deficiency in the lipoprotein lipase (LPL) enzyme that hydrolyzes triglycerides in chylomicrons and very low-density LDL particles. In contrast to the action of LPL, ApoC3 inhibits the clearance of triglyceride-rich lipoproteins via inhibition of LPL and LPL-independent mechanisms.³⁵ In a phase 3 study of patients with FCS, volanesorsen decreased ApoC3 levels by 84% from baseline. Notably, total triglyceride fell 77% from mean 2267 ± 1259 mg/dL at baseline to 590 ± 497 mg/dL at 3 months with once-weekly subcutaneous injections.³⁶ While the decrease in triglycerides was significant, almost 50% of patients developed a fall in platelet counts below 100 000 per microliter, and 2 patients' counts fell below 25 000, leading to treatment discontinuation.³⁶ In the United States (US), volanesorsen has not been granted Food and Drug Administration approval. A phase 2/3 study of volanesorsen for the treatment of familial partial lipodystrophy and a phase 2 study of a second-generation ApoC3 GalNAc-ASO conjugate for the treatment of

hypertriglyceridemia in patients with cardiovascular disease are ongoing (Table 1).

Inclisiran, a GalNAc-siRNA conjugate that suppresses PCSK9 synthesis, significantly reduced LDL-C levels in large, phase 3 trials of patients with familial hypercholesterolemia, atherosclerotic cardiovascular disease (ASCVD), or high risk of ASCVD.^{20,21} LDL receptors on the hepatocyte cell surface remove LDL-C from circulation, following which the receptor is recycled back to the cell surface. Secreted by the liver, PCSK9 regulates the number of LDL receptors by binding them and thus diverting them from recycling to degradation. Gain-of-function variants of PCSK9, hence, lower LDL receptor numbers resulting in higher LDL-C and cardiovascular risk.³⁷ Loss-of-function variants have the opposite effect.²²

Inclisiran has a sustained duration of action and potency. In ORION-1, a phase 2 study of patients with ASCVD and elevated LDL-C (on maximally tolerated

doses of statins), a single 300-mg subcutaneous dose lowered LDL-C by 38.4% from baseline on day 180. A second dose (day 90) resulted in a 52.6% decrease from baseline on day 180.³⁸ Data from phase 3 ORION-10 and ORION-11 trials has validated these results.²¹ These 2 studies enrolled patients with ASCVD or an ASCVD equivalent (type 2 diabetes, familial hypercholesterolemia, or a 10-year risk of a cardiovascular event of $\geq 20\%$ as assessed by Framingham Risk Score for Cardiovascular Disease or equivalent), who had a baseline LDL-C of ≥ 70 mg/dL (100 mg/dL for ASCVD equivalent) on a maximally tolerated dose of statins.²¹ Administered every 6 months, inclisiran reduced LDL-C by 52.3% and 49.9% on day 510 in ORION-10 and ORION-11, respectively.²¹ ORION-9 tested the same regimen in patients with heterozygous familial hypercholesterolemia. On day 510, inclisiran reduced LDL-C by 39.7% from a baseline level of 151.4 ± 50.4 mg/dL.²⁰ Adverse events were similar between the inclisiran and placebo groups in ORION-9, -10, and -11. Injection-site reactions were more common in the inclisiran group and were generally mild. ORION-4, an ongoing trial of 15 000 patients, will assess the impact of inclisiran on cardiovascular outcomes.³⁹ Together, the results indicate that, if approved, inclisiran has the potential to be a novel, safe, effective approach to treating hypercholesterolemia in high-risk patients. The pharmacodynamic profile supporting infrequent (biannual) administration additionally suggests this approach could minimize patient noncompliance with treatment and allow hypercholesterolemia to be managed with a dosing strategy more akin to routine vaccinations for diseases such as influenza. Approval of inclisiran is under review in the US and Europe.

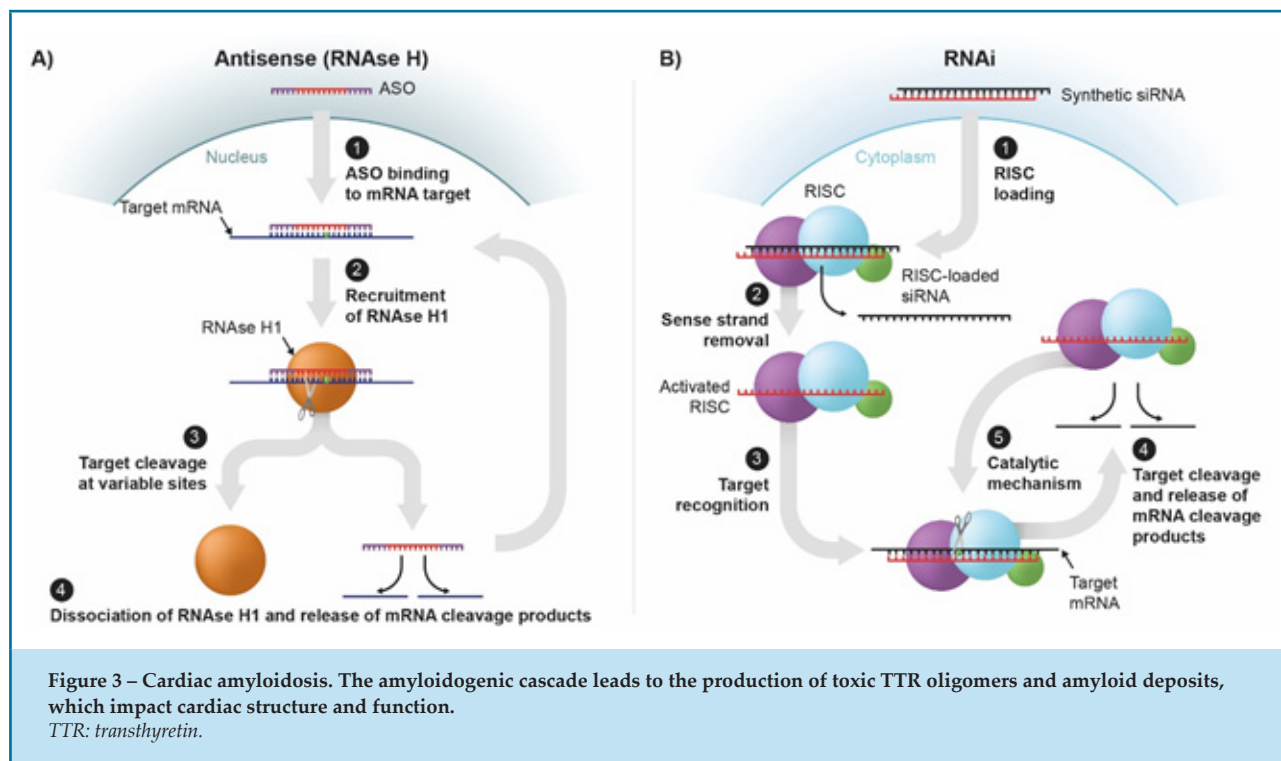
Gene silencing in transthyretin-mediated amyloidosis

Transthyretin-mediated (ATTR) amyloidosis is a rare, underdiagnosed, rapidly progressive, debilitating, and fatal disease caused by misfolded transthyretin (TTR) (prealbumin) that accumulates as amyloid deposits in multiple tissues.^{40,41} Cardiomyopathy and polyneuropathy are the most prominent features, but systemic manifestations arise from amyloid infiltration of other tissues, including the gastrointestinal (GI) tract.^{41,42} Polyneuropathy includes sensory, motor, and autonomic impairment and has an aggressive course leading to deteriorating quality of life (QOL) and loss of function.⁴¹ Cardiac involvement typically presents as heart failure with preserved ejection fraction (HFpEF) or unexplained left ventricular (LV) wall thickening;⁴³ progressive

symptoms of heart failure (HF) and cardiac arrhythmias are experienced, with death typically occurring 2.5-5.5 years after cardiomyopathy diagnosis.^{44,45} In 2 studies, 13% of patients with HFpEF and 16% of older patients who underwent transcatheter aortic valve replacement for severe aortic stenosis had evidence of cardiac amyloid involvement.^{46,47} While delays in diagnosis are common,⁴⁸ recognition of the early signs of ATTR amyloidosis (e.g., connective tissue manifestations such as carpal tunnel syndrome)⁴³ could be an important opportunity for therapeutic intervention before cardiac (and/or neurologic) symptoms develop.⁴⁹

Pharmacotherapies that target steps in the amyloidogenic pathway are available. TTR is predominantly produced in the liver and normally circulates as a stable tetramer. However, abnormal destabilization of the protein causes dissociation into monomers that misfold, deposit, and accumulate as amyloid in various tissues, including heart (Figure 3), nerves, and GI tract.⁵⁰ The disease has 2 types, hereditary and wild-type (hATTR and wtATTR amyloidosis, respectively).^{40,41} In hATTR amyloidosis, also known as ATTRv amyloidosis, *TTR* gene variants destabilize the protein.⁵⁰ hATTR amyloidosis can have a heterogeneous presentation, differing according to *TTR* variant, age of onset, and geography.⁵¹ Historically, certain variants have been associated with predominant cardiomyopathy or neuropathy; however, a majority of patients develop a mixed phenotype.⁵²⁻⁵⁴ Several geographies are endemic for hATTR amyloidosis, including Brazil, where the most common variant, V30M, was found in 92% of patients.⁵⁵ This variant has previously been associated with predominant polyneuropathy, although a Brazilian study reported cardiac symptoms in at least 40% of patients with ATTRV30M amyloidosis.⁵⁶ In contrast, patients with wtATTR amyloidosis do not carry a *TTR* variant and disease is associated with aging;⁵⁷ patients typically present with predominant cardiomyopathy.⁵⁸

Orthotopic liver transplantation (OLT) was the first available treatment for patients with hATTR amyloidosis with polyneuropathy, and it acts by eliminating the production of variant TTR.⁵⁹ In patients with early-stage polyneuropathy and absence of cardiac involvement, certain symptoms can improve or stabilize in the short term following OLT,⁵⁹ supporting the therapeutic rationale for silencing the *TTR* gene in this disease. However, disease progression can occur after OLT; death of 38% of patients due to cardiac events post OLT was reported in a retrospective study.⁶⁰ End-stage HF and



sudden death have been observed, due to continued deposition of wild-type (wt) TTR at the site of pre-existing cardiac deposits (e.g., in the myocardium).^{59,61}

Thyroid hormone binding to TTR stabilizes the tetramer,⁶² motivating the development of small-molecule stabilizers (e.g., diflunisal and tafamidis). Diflunisal, a nonsteroidal anti-inflammatory therapeutic, slows the worsening of neuropathy in patients with hATTR amyloidosis.⁶³ However, adverse effects on renal and platelet function have limited its use in patients with cardiomyopathy,⁶⁴ and diflunisal is not approved in any region for the treatment of ATTR amyloidosis. Tafamidis can delay peripheral neurologic impairment⁶⁵ and is available for the treatment of early-stage neuropathy in regions including Latin America (e.g., Brazil, Mexico), EU, and Japan.^{66,67} Tafamidis has not been approved in the US as a treatment for hATTR amyloidosis with polyneuropathy. Tafamidis has, however, been recently approved for treatment of ATTR amyloidosis with cardiomyopathy following a study of patients with wtATTR and hATTR amyloidosis with New York Heart Association class 1, 2, or 3 HF.⁶⁸ At 30 months, tafamidis was associated with lower all-cause mortality than placebo (hazard ratio [HR], 0.70; 95% confidence interval [CI], 0.51-0.96) and a lower rate of cardiovascular-related hospitalizations (relative risk ratio 0.68 per year; 95% CI,

0.56-0.81). Secondary analyses demonstrated lower rates of decline in 6-minute walk test (6-MWT) distance and in Kansas City Cardiomyopathy Questionnaire overall score compared with placebo.⁶⁸

Phase 3 studies of 2 oligonucleotide therapeutics, inotersen and patisiran, validated TTR gene silencing in the liver as an effective therapeutic strategy in hATTR amyloidosis with polyneuropathy^{53,54} and offered insights into the potential of this approach in patients with cardiomyopathy. Indeed, patisiran and 2 other oligonucleotide therapeutics are being evaluated in ongoing trials for ATTR amyloidosis with cardiomyopathy (Table 1).

The efficacy of inotersen and patisiran was evaluated via 2 measures in the pivotal studies of patients with hATTR amyloidosis with polyneuropathy: the modified Neuropathy Impairment Score+7 tests (mNIS+7), a composite measure of neuropathy (higher scores indicate more impairment⁶⁹); and the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire, which assesses patients' perception of their QOL (higher scores indicate poorer QOL⁷⁰). Of note, the scoring range of mNIS+7 differed between the trials of inotersen (-22.4-346.3) and patisiran (0-304), reflecting differences in the measurement of sensation, nerve conduction, and autonomic function.^{53,54}

Inotersen is an ASO administered weekly via subcutaneous injection. In the phase 3 NEURO-TTR trial, inotersen reduced serum TTR protein levels to a median nadir of 79% from baseline.⁵⁴ This resulted in significant improvement compared with placebo in mNIS+7 scores (least-squares mean change from baseline on week 66 of +5.8 points in the inotersen group compared with +25.5 points in the placebo group) and in Norfolk QOL-DN scores (mean change from baseline was +1.0 and +12.7 points for inotersen and placebo groups, respectively).⁵⁴

Among patients in the NEURO-TTR trial, 63% had echocardiographic signs of cardiac amyloid involvement, but no significant changes were observed in echocardiographic variables at the end of the study.⁵⁴ A small, uncontrolled study of inotersen for patients with hATTR or wtATTR amyloidosis with cardiomyopathy suggested disease improvement relative to baseline after 1-3 years of treatment, with declines in LV mass, LV wall thickness, and *N*-terminal prohormone of brain-type natriuretic peptide (NT-proBNP), as well as an improvement in global longitudinal strain (GLS).⁷¹ Functional capacity appeared to stabilize or improve relative to baseline over the first 2 years of treatment in patients with hATTR amyloidosis, as quantified by the 6-MWT.⁷¹ In contrast, data from natural history studies show a progressive decline in the 6-MWT over a similar period.^{44,45}

Inotersen is approved in several countries, including Brazil, for hATTR amyloidosis with polyneuropathy, but treatment requires regular monitoring for thrombocytopenia and acute glomerulonephritis. Each complication occurred in 3% of patients in NEURO-TTR; 1 death was associated with a case of grade 4 thrombocytopenia.⁵⁴ Inotersen is not being developed for cardiomyopathy; however, a GalNAc-ASO conjugate with a similar sequence and design to inotersen is being evaluated in a phase 3 trial of patients with hATTR or wtATTR amyloidosis with cardiomyopathy (Table 1).

Patisiran, an siRNA formulated in an LNP, is administered intravenously every 3 weeks. In the phase 3 APOLLO trial, patisiran reduced TTR protein levels by median 81% from baseline.⁵³ Patisiran resulted in improvements in mNIS+7 and Norfolk QOL-DN scores relative to both placebo and to the patients' own baseline.⁵³ In the patisiran and placebo arms, the least-squares mean changes from baseline were -6.0 and +28.0 in mNIS+7 and -6.7 and +14.4 in Norfolk QOL-DN, respectively. Patisiran also improved gait speed in a 10-meter walk test (change relative to baseline was +0.08

and -0.24 meters per second in the patisiran and placebo arms, respectively).⁵³

Data from APOLLO suggest that patisiran may also improve measures of cardiac structure and function in patients with cardiomyopathy. Prespecified exploratory analyses assessed patisiran and placebo in a subpopulation with evidence of cardiac amyloid involvement, defined by a baseline LV wall thickness ≥ 13 mm and no history of aortic valve disease or hypertension.⁷² Reflecting the multisystem nature of hATTR amyloidosis, the cardiac subpopulation comprised 56% of APOLLO patients who were enrolled on the basis of polyneuropathy. Compared with placebo, patisiran reduced mean LV wall thickness, increased end-diastolic volume, decreased GLS, increased cardiac output, and lowered NT-proBNP. A decrease in GLS in the patisiran arm is notable since an absolute 1% increase has previously been associated with an increased risk of death (HR, 1.1, 95% CI, 1.01-1.19).⁷³ Patisiran demonstrated a positive benefit:risk profile in both the overall and cardiac subpopulations. In post hoc analyses of safety data, among the entire APOLLO population, the exposure-adjusted rates of cardiac hospitalizations and all-cause death were 18.7 and 10.1 per 100 patient-years in the placebo and patisiran groups, respectively (Andersen-Gill HR, 0.54; 95% CI, 0.28-1.01).⁷² While these data suggest that improvement in cardiac parameters following patisiran treatment may lead to improved outcomes, the APOLLO study was not designed to investigate clinical outcomes such as death or cardiovascular hospitalization, and further studies are needed.

Patisiran is approved in > 30 countries globally, including Brazil, for the treatment of hATTR amyloidosis with polyneuropathy (specific indications vary by country/region).⁷⁴⁻⁷⁸ Except for infusion-related reactions, the overall incidence and types of adverse effects were similar between patisiran and placebo arms of the APOLLO trial. Infusion-related reactions occurred in 19% and 9% of patisiran- and placebo-treated patients, respectively.⁵³ They were generally mild or moderate, spontaneously resolved, and decreased in frequency with subsequent infusions; risk was reduced by premedicating patisiran-treated patients. An ongoing trial, APOLLO-B, is evaluating the safety and efficacy of patisiran in ATTR amyloidosis with cardiomyopathy (Table 1).

Vutrisiran, a second-generation investigational RNAi therapeutic targeting TTR, uses a GalNAc-siRNA ESC conjugate, similar to that used for inclisiran, with chemical modifications that confer increased potency and

high metabolic stability. Administered subcutaneously once every 3 months in a phase 1 study of healthy volunteers, a single 25-mg dose resulted in a maximum 80% TTR reduction sustained for approximately 90 days.⁷⁹ This vutrisiran regimen is predicted to achieve 88-90% TTR reduction, similar to patisiran in the APOLLO trial. Two phase 3 trials are currently ongoing to evaluate vutrisiran for the treatment of hATTR amyloidosis with polyneuropathy (HELIOS-A) and ATTR amyloidosis with cardiomyopathy (HELIOS-B; Table 1).

Summary

More than 40 years after Zamecnik envisioned targeting RNA as a therapeutic modality, oligonucleotides have emerged as cutting-edge medicines that exploit endogenous mRNA degradation mechanisms. By silencing the expression of disease-causing proteins with genetic precision, siRNAs and ASOs expand the range of biologic targets beyond those that require direct drug-protein binding. Advances in nucleic acid chemistry and oligonucleotide delivery to the liver have yielded novel therapeutic approaches to cardiovascular diseases. They include common conditions like hypercholesterolemia and rarer ones like ATTR amyloidosis, which may be more prevalent than previously thought. GalNAc-siRNA conjugates have demonstrated positive benefit:risk profiles in clinical studies and have potential to be convenient treatments for conditions that are difficult to manage. Their characteristics also raise the possibility that these therapeutics may be useful for both symptomatic and presymptomatic individuals. For example, early diagnosis and quarterly treatment with an siRNA in patients with noncardiac signs of

ATTR amyloidosis may prevent the development of congestive HF. Also, semiannual injections of inclisiran for high-risk individuals with hypercholesterolemia may achieve better outcomes than current medications whose compliance is inconsistent. While oligonucleotide-based therapeutics are relatively new, their ability to target the fundamental bases of diseases is already clear, and their impact on medicine is certain to grow.

Author contributions

Conception and design of the research: Jay PY, Vest J. Acquisition of data: Jay PY, Maier MA. Analysis and interpretation of the data: Jay PY. Writing of the manuscript: Jay PY, Maier MA. Critical revision of the manuscript for intellectual content: all authors.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Erratum

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