




## REVIEW

## Liver-directed drugs for transthyretin-mediated amyloidosis

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## Abstract

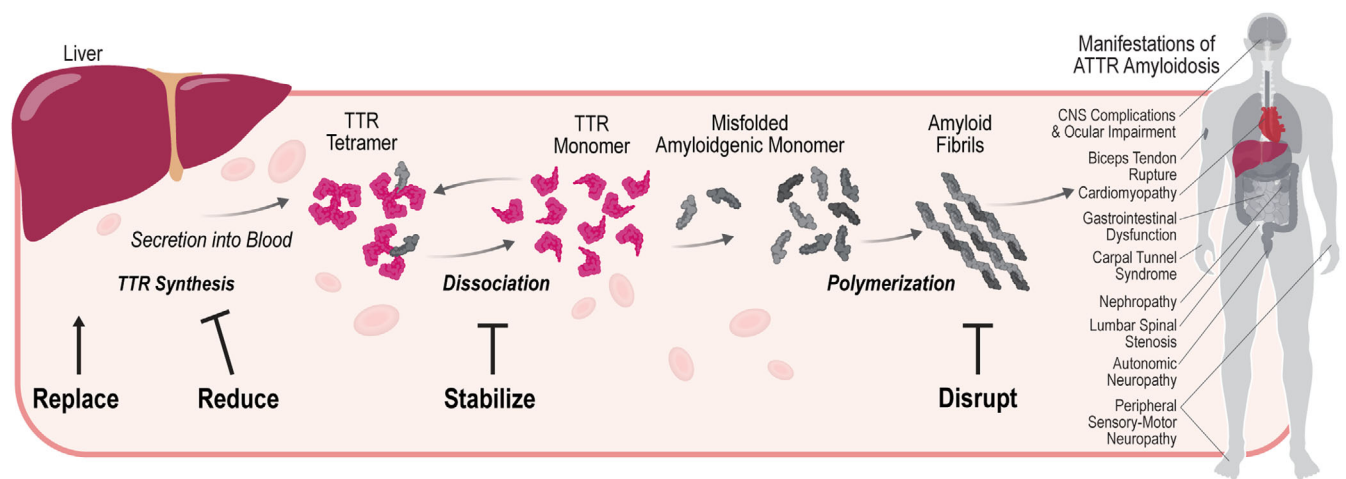
Transthyretin-mediated amyloidosis (ATTR) is a rare, under-recognized, progressively debilitating, fatal disease caused by the aggregation and extracellular deposition of amyloid transthyretin (TTR) fibrils in multiple organs and tissues throughout the body. TTR is predominantly synthesized by the liver and normally circulates as a homotetramer, while misfolded monomers aggregate to form amyloid fibrils. One strategy to treat ATTR amyloidosis is to reduce the amount of TTR produced by the liver using drugs that directly target the *TTR* mRNA or gene. This narrative review focuses on how TTR gene silencing tools act to reduce TTR production, describing strategies for improved targeted delivery of these agents to hepatocytes where TTR is preferentially expressed. Antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs), termed RNA silencers, cause selective degradation of *TTR* mRNA, while a *TTR* gene editing tool reduces TTR expression by introducing nonsense mutations into the *TTR* gene. Two strategies to facilitate tissue-specific delivery of these nucleic acid-based drugs employ endogenous receptors expressed by hepatocytes. Lipid nanoparticles (LNPs) that recruit apolipoprotein E support low-density lipoprotein receptor-mediated uptake of unconjugated siRNA and are now used for CRISPR gene editing tools. Additionally, conjugating N-acetylgalactosamine (GalNAc) moieties to ASOs or siRNAs facilitates receptor-mediated uptake by the asialoglycoprotein receptor. In summary, ATTR is a progressive disease with various clinical manifestations due to TTR aggregation, deposition, and amyloid formation. Receptor-targeted ligands (eg, GalNAc) and nanoparticle encapsulation (eg, LNPs) are technologies to deliver ASOs, siRNAs, and gene editing tools to hepatocytes, the primary location of TTR synthesis.

## KEYWORDS

amyloid, antisense therapy, ATTR, cardiomyopathy, CRISPR, gene editing, polyneuropathy, RNAi, silencer, siRNA

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**FIGURE 1** Approaches to Transthyretin-mediated Amyloidosis Treatment. The following are treatment modalities to replace, reduce, stabilize, or disrupt transthyretin (TTR) in the treatment of transthyretin-mediated amyloidosis (ATTR). Replace TTR: liver transplant. Reduce TTR: ASO (inotersen, eplontersen); siRNA (patisiran, vutrisiran); Gene editing (NTLA-2001). Stabilize TTR: tafamidis, acoramidis, diflunisal [off-label use]. Disrupt amyloid fibrils: Combination therapy (doxycycline and tauroursodeoxycholic acid); Monoclonal antibodies (PRX004, NI006); Pan-amyloid technologies (AT-02, AT-03, AT-04 [all preclinical]).

## 1 | TRANSTHYRETIN AMYLOIDOSIS

### 1.1 | Transthyretin

Transthyretin-mediated amyloidosis (ATTR) is a rare and progressive disease that reduces survival and quality of life. ATTR is caused by the deposition of transthyretin (TTR) amyloid fibrils in various organs and tissues.<sup>1-3</sup> TTR is encoded by a single gene on chromosome 18, and though conserved across species, it is thought to be a non-essential protein.<sup>4</sup> The TTR protein is abundant, predominantly synthesized by the liver and secreted to the plasma, though a small amount of TTR is synthesized by the retinal pigment epithelium, pancreas, and choroid plexus (<5% of the total combined). TTR is responsible for the transport of the retinol vitamin A and thyroxine.<sup>1,5,6</sup>

TTR normally circulates as a homo-tetramer in equilibrium with component homodimers and monomers. TTR monomers are susceptible to misfolding. In patients with ATTR, misfolded TTR monomers (both full-length and fragmented) aggregate and form fibrils that are deposited in systemic tissues and organs (Figure 1).<sup>3,7-9</sup>

### 1.2 | ATTR

There are two forms of ATTR. The hereditary form of ATTR (ATTRv) is due to the aggregation of variant TTR in amyloid fibrils, while wild-type ATTR (wtATTR) is due to aggregation of normal TTR—a process that occurs with advancing age. Most ATTRv patients experience a combination of polyneuropathy (PN) and cardiomyopathy (CM) symptoms whereas wtATTR primarily affects the heart.

There are >130 single-point mutations in the TTR gene that are associated with ATTRv.<sup>10,11</sup> Some genetic variants principally induce a PN phenotype and others manifest a CM phenotype; however, the

majority of TTR variants result in a mixed phenotype with both cardiac and neurologic involvement.<sup>12,13</sup> Other clinical manifestations of ATTR include gastrointestinal, renal, bladder, ocular, central nervous system (CNS), and autonomic nervous system (orthostasis, erectile) dysfunctions.<sup>10,14</sup> In addition to phenotypic differences, TTR mutations can vary in terms of population prevalence and geographic distribution.<sup>11,15</sup> Phenotypes and disease severity are influenced by factors such as age of onset, gender, and paternal or maternal inheritance.<sup>15,16</sup>

Polyneuropathy associated with ATTRv (ATTRv-PN) includes both early- and late-onset phenotypes. It is characterized by amyloid fibril deposition in the endoneurium and surrounding blood vessels.<sup>17,18</sup> In the early stages of ATTRv-PN, the fibrils tend to accumulate in peripheral small nerve fibers, resulting in distal sensory symptoms of pain, numbness, and paresthesia.<sup>13,14,19</sup> Autonomic dysfunctions such as gastrointestinal issues and erectile dysfunction can also occur, along with more general symptoms such as weight loss and fatigue. As the disease progresses, sensory symptoms extend proximally and patients usually experience loss of sensation and reflexes, muscle weakness, steppage gait, and impaired balance. Cardiac manifestations are not always apparent at diagnosis, although they are often found at presentation in males with late-onset ATTRv.<sup>13</sup> For patients with ATTRv-PN, survival time from symptom onset is approximately 6–12 years, with cardiac involvement and/or multiple organ failure usually contributing to mortality.<sup>13,20</sup>

Wild-type TTR amyloid cardiomyopathy (wtATTR-CM) is diagnosed predominantly (~85%) in men  $\geq 60$  years of age.<sup>10</sup> The hereditary form of cardiomyopathy (ATTRv-CM) may present much earlier (from late 20s to early 40s), affecting both men and women equally.<sup>21</sup> Both types are characterized by the accumulation of amyloid fibrils in the myocardium, which leads to thickening of ventricular walls, conduction abnormalities, arrhythmias, and heart failure.<sup>22,23</sup> Diagnosis

**TABLE 1** TTR gene silencing agents

Nucleic acid target	Gene silencer <sup>a</sup>	Drug name	Formulation	Ligand conjugate	Admin route	Dose	Indication(s)	Phase <sup>b</sup>
RNA	ASO	Inotersen	-	-	SC	284 mg QW	ATTRv-PN	Approved
		Eplontersen <sup>c</sup>	-	GalNAc	SC	45 mg Q4W	ATTRv-PN ATTR-CM	Phase 3
	siRNA	Patisiran	LNP	-	IV Infusion	0.3 mg/kg Q3W <sup>d</sup>	ATTRv-PN	Approved
		Revusiran	-	GalNAc	SC	500 mg QW	ATTR-CM	D/C
		Vutrisiran <sup>e</sup>	-	GalNAc	SC	25 mg Q3M	ATTRv-PN ATTR-CM	Approved Phase 3
DNA	CRISPR-Cas9	NTLA-2001	LNP	-	IV Infusion	Up to 1 mg/kg single, one-time dose <sup>f</sup>	ATTRv-PN ATTR-CM	Phase 1

Abbreviations: ASO, antisense oligonucleotide; ATTR(v), (hereditary) transthyretin amyloidosis; CM, cardiomyopathy; CRISPR, gene editing technology (targeted gene knockout); D/C, discontinued; GalNAc, triantennary N-acetylgalactosamine; IV, intravenous; LNP, lipid nanoparticle; PN, polyneuropathy; SC, subcutaneous; siRNA, small interfering RNA (ribonucleic acid); Q3M, every 3 months; Q3W, every 3 weeks; Q4W, every 4 weeks; QW, every week.

<sup>a</sup>ASO leads to RNase-H1-mediated mRNA degradation, siRNA leads to Ago2-mediated mRNA degradation, and CRISPR-Cas9 leads to DNA gene editing. <sup>b</sup>As of May 2022.

<sup>c</sup>Eplontersen also known as ION-682884, IONIS-TTR-LRx, and AKCEA-TTR-LRx.

<sup>d</sup>Dosage for patients <100 kg; dosage is 30 mg for those ≥100 kg;

<sup>e</sup>Vutrisiran also known as ALN-TTRsc02.

<sup>f</sup>Ascending dose trial underway.

can be challenging since these symptoms overlap with more common cardiac conditions.<sup>24</sup> However, certain clinical manifestations may be considered “clues” or “red flags” for ATTR-CM, including bilateral carpal tunnel syndrome, polyneuropathy, trigger finger, tendon rupture, lumbar spinal stenosis, intolerance to standard heart failure medications, and autonomic dysfunctions (eg, gastrointestinal problems, orthostatic hypotension).<sup>12,14</sup> In contrast to patients with ATTR-PN, ATTR-CM can be fatal within 2–6 years after diagnosis,<sup>12,24</sup> depending on the stage of the disease.

## 2 | ATTR TREATMENT STRATEGIES

Until recently, the standard of care for patients with ATTRv was to *replace* the factory of mutant TTR through liver transplantation. However, this leaves recipients with a threat of organ rejection, a lifetime of immunosuppressant medications with attendant risks of infection and malignancy, and the possibility of progressive wild-type TTR amyloid fiber accumulation.<sup>25,26</sup> Alternative strategies that are approved or are in various stages of development overcome the challenges associated with organ transplantation and have the added potential to treat patients with either wild type or hereditary ATTR. These approaches act at either the nucleic acid level (gene silencers) or at the protein level (protein stabilizers or disruptors) (Figure 1).<sup>10,27,28</sup> Specifically, RNA silencers and gene editing tools are designed to act at the nucleic acid level to *reduce* TTR synthesis by the liver. Small molecule drugs are designed to act at the protein level to *stabilize* the TTR tetramer and limit the rate of dissociation to monomers and consequent amyloid formation.<sup>10,11,29</sup> Agents that *disrupt* pre-existing amyloid fibrils promote their removal by macrophages.<sup>10</sup> The mechanism by which gene silencers reduce serum TTR and strategies to facilitate their selective delivery to hepatocytes are discussed below.

## 3 | REDUCTION OF SERUM TTR LEVELS WITH RNA SILENCERS OR GENE EDITING TOOLS

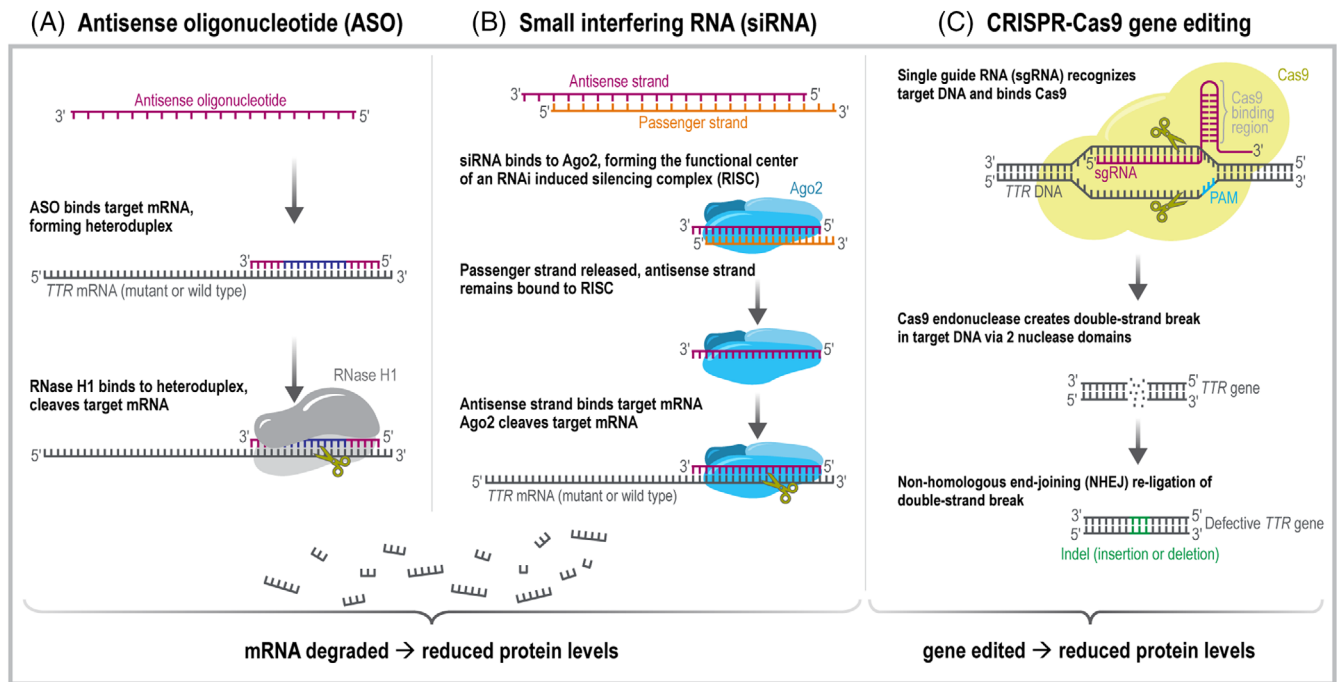
RNA silencers and gene editing tools target the *TTR* mRNA or gene, respectively, to reduce circulating serum TTR levels.<sup>30</sup> Current agents approved or under investigation in the clinic include the antisense oligonucleotides (ASOs) inotersen and eplontersen; the small interfering RNAs (siRNAs) patisiran and vutrisiran; and a CRISPR/Cas9 gene editing tool NTLA-2001 (Table 1; Figure 2).<sup>3,10,31</sup> Due to the role of TTR as a transporter via binding to retinol-binding protein, vitamin A supplementation is recommended with siRNA and ASO treatments.<sup>32,33</sup>

### 3.1 | RNA silencers: antisense oligonucleotides and siRNAs to reduce serum TTR

ASOs and siRNAs are designed to alter the disease phenotype of ATTR amyloidosis by degrading both wild-type and mutant *TTR* RNA transcripts, reducing the synthesis of TTR protein.<sup>10</sup> They employ short sequences of synthetic oligonucleotides to specifically bind the *TTR* mRNA through Watson-Crick base pair interactions and support endogenous ribonuclease activity.<sup>34,35</sup> The single-stranded (ASOs) or double-stranded (siRNAs) synthetic oligonucleotides generally range in length from 15 to 30 nucleotides and are chemically modified to increase affinity for the target RNA and impart greater resistance to degradation by nucleases as well as to limit off-target effects (Table 2).<sup>5,30,36–39</sup>

#### 3.1.1 | Antisense oligonucleotides

The two ASO treatments that have been evaluated in ATTR amyloidosis, inotersen and eplontersen, have identical sequences (Table 1).



**FIGURE 2** Mechanisms of RNA Silencing and DNA-Editing Treatments for ATTR. Panel A: Single-stranded ASOs (eg, inotersen and eplontersen) consist of 10 DNA nucleotides flanked by five chemically modified ribonucleotides. The ASO binds target *TTR* mRNA, forming a DNA/RNA heteroduplex that binds to and activates ribonuclease H1 (RNase H1). RNase H1 then cleaves the target mRNA. *TTR* mRNA is degraded, reducing expression of mutant and wild-type *TTR*. Panel B: Double-stranded siRNA (eg, patisiran, vutrisiran) is chemically modified to prevent degradation and is composed of an antisense strand (complementary to the target mRNA) and a passenger strand. The siRNA binds argonaute2 (Ago2) and other proteins to form the RNA-induced silencing complex (RISC). Once assembled, the passenger strand is released. The antisense strand remains bound to RISC and binds to target *TTR* mRNA, which is then cleaved by Ago2. *TTR* mRNA is degraded, reducing expression of mutant and wild-type *TTR*. Panel C: CRISPR-Cas 9 gene editing tool (eg, NTLA-2001) consists of a single guide RNA (sgRNA) and a Cas9 mRNA sequence (which is translated into Cas9 endonuclease protein once inside the cell). The upstream region of sgRNA contains a 20-nucleotide sequence that is complementary to *TTR* DNA and is guided there by a 3-nucleotide protospacer adjacent motif (PAM). The downstream region of sgRNA binds to Cas9. After cleavage of the *TTR* DNA via two nuclease domains of Cas9, the double-strand break is re-ligated via non-homologous end-joining (NHEJ). NHEJ is likely to generate insertion and deletion errors (indels) in the re-ligated DNA strands, which effectively decreases functional *TTR* mRNA levels and subsequent protein production.

They bind directly to the 3' untranslated region (UTR) of the *TTR* mRNA where there are no known mutations<sup>40,41</sup> and contain ten continuous 2'-deoxynucleotides flanked by five chemically modified ribonucleotides. A DNA/RNA heteroduplex forms upon ASO binding to the mRNA which activates RNase H1 to cleave the *TTR* mRNA, thereby reducing expression of both the mutant and wild-type *TTR* protein (Figure 2A).<sup>30,38,40-42</sup>

Inotersen has been approved for the treatment of polyneuropathy in adult patients with ATTRv amyloidosis in the US, EU, Canada, UK, and Brazil (stage 1 or 2 only by the European Medicines Agency [EMA]).<sup>33,43,44</sup> In patients with ATTRv-PN, treatment with inotersen slowed the progression of the disease as measured by a neuropathy impairment score and reduced the decline in quality of life over time.<sup>45,46</sup> Because of an increased risk of glomerulonephritis and thrombocytopenia, renal function and platelet count safety monitoring is required in patients receiving inotersen.<sup>33</sup>

Eplontersen is a ligand-conjugated antisense oligonucleotide (LICA) with a high-affinity ligand, *N*-acetylgalactosamine (GalNAc), covalently attached to the amino terminus to facilitate uptake by a

tissue-specific receptor, asialoglycoprotein receptors (ASGPR), in hepatocytes. In a phase 1 clinical trial in healthy volunteers, eplontersen demonstrated an approximately 30-fold increase in potency in reducing *TTR* levels compared to inotersen.<sup>40</sup> No severe or serious adverse events, and no significant abnormalities in renal or hematological parameters were reported. Phase 3 clinical trials are currently underway to determine the effect of eplontersen on disease course in patients with ATTRv-PN and all ATTR-CM.<sup>40,47-49</sup>

### 3.1.2 | Small interfering RNAs

Similar to ASOs, the three siRNAs that have been evaluated for ATTR, patisiran, revusiran and vutrisiran, are designed to target the 3'UTR of the *TTR* mRNA (Table 1).<sup>50,51</sup> Each siRNA is composed of an antisense strand complementary to the *TTR* mRNA and a passenger strand, and is chemically modified to protect the molecule from degradation.<sup>39</sup> Once in the cytoplasm, the siRNA binds to the protein argonaute2 (Ago2) forming the functional center of the RNA-induced silencing

**TABLE 2** Comparison of ASO and siRNA properties

Similarities	ASO and siRNA	
Structure	<ul style="list-style-type: none"> <li>• 15-30 nucleotides long</li> </ul>	
mRNA binding	<ul style="list-style-type: none"> <li>• Binds target mRNA through Watson-Crick base pairing</li> </ul>	
Nucleotide modifications	<ul style="list-style-type: none"> <li>• Chemical modifications to nucleotide subunits (phosphate group backbone, ribose sugar, and base) for improved stability, increased potency and affinity to target transcripts, improved bioavailability, decreased renal clearance, and/or reduced toxicity/immunogenicity</li> </ul>	
Dosing	<ul style="list-style-type: none"> <li>• Repeated doses required</li> </ul>	
Differences	ASO	siRNA
Structure	<ul style="list-style-type: none"> <li>• Flexible, single-stranded</li> <li>• Hydrophilic and hydrophobic → can bind to many proteins</li> </ul>	<ul style="list-style-type: none"> <li>• Rigid, double-stranded (antisense strand [binds target DNA] + passenger strand)</li> <li>• Hydrophilic → decreased cellular uptake and reduced protein binding</li> </ul>
mRNA cleavage	<ul style="list-style-type: none"> <li>• via RNase H1</li> </ul>	<ul style="list-style-type: none"> <li>• via Ago2 (within the RISC complex)</li> </ul>
Non-nucleotide modifications for systemic delivery	<ul style="list-style-type: none"> <li>• Not required, but can be conjugated for targeted cellular delivery (eg, GalNAc)</li> </ul>	<ul style="list-style-type: none"> <li>• Requires encapsulation (eg, LNP) or binding to a conjugate (eg, GalNAc) for cellular delivery</li> </ul>

Abbreviations: Ago2, argonaute 2; ASO, antisense oligonucleotide; PS, phosphorothioate (modification to phosphate backbone); RISC, RNA-induced silencing complex; siRNA, small interfering RNA.

complex (RISC).<sup>52-54</sup> After assembly, the siRNA unwinds and the functional antisense strand remains bound to RISC while the passenger strand is released. The now single-stranded antisense strand binds to *TTR* mRNA and the mRNA is cleaved by Ago2 and ultimately degraded, leading to reductions in production of both mutant and wildtype *TTR* protein (Figure 2B).<sup>5,34,39,55</sup>

Patisiran is a lipid nanoparticle (LNP) encapsulated siRNA that has been approved for the treatment of polyneuropathy of adult patients with hereditary ATTR amyloidosis in the US, EU, Canada, UK, Brazil, Japan and Israel (stage 1 or 2 only by the EMA). In patients with ATTRv-PN, patisiran was effective in treating disease progression.<sup>56</sup> Patisiran treatment requires premedication with a corticosteroid and antihistamine due to infusion-related reactions.<sup>32</sup>

Revusiran was the first GalNAc-siRNA that was investigated in the clinic for ATTR.<sup>37,57</sup> Weekly dosing with revusiran showed a dose-dependent reduction in *TTR* levels.<sup>57-59</sup> However, clinical development of revusiran was halted due to a mortality imbalance between the revusiran and placebo treatment arms, though no clear causative mechanism for the increased deaths was identified.<sup>59,60</sup>

Vutrisiran is a GalNAc conjugated siRNA approved for the treatment of ATTRv-PN in the US. In the registrational phase 3 trial involving patients with ATTRv-PN, the study endpoints were met and vutrisiran was effective in treating multiple disease-related outcomes relative to placebo.<sup>61,62</sup> A phase 3 trial in patients with ATTR-CM is ongoing.<sup>63</sup>

### 3.2 | CRISPR-Cas gene editing tool to reduce serum TTR

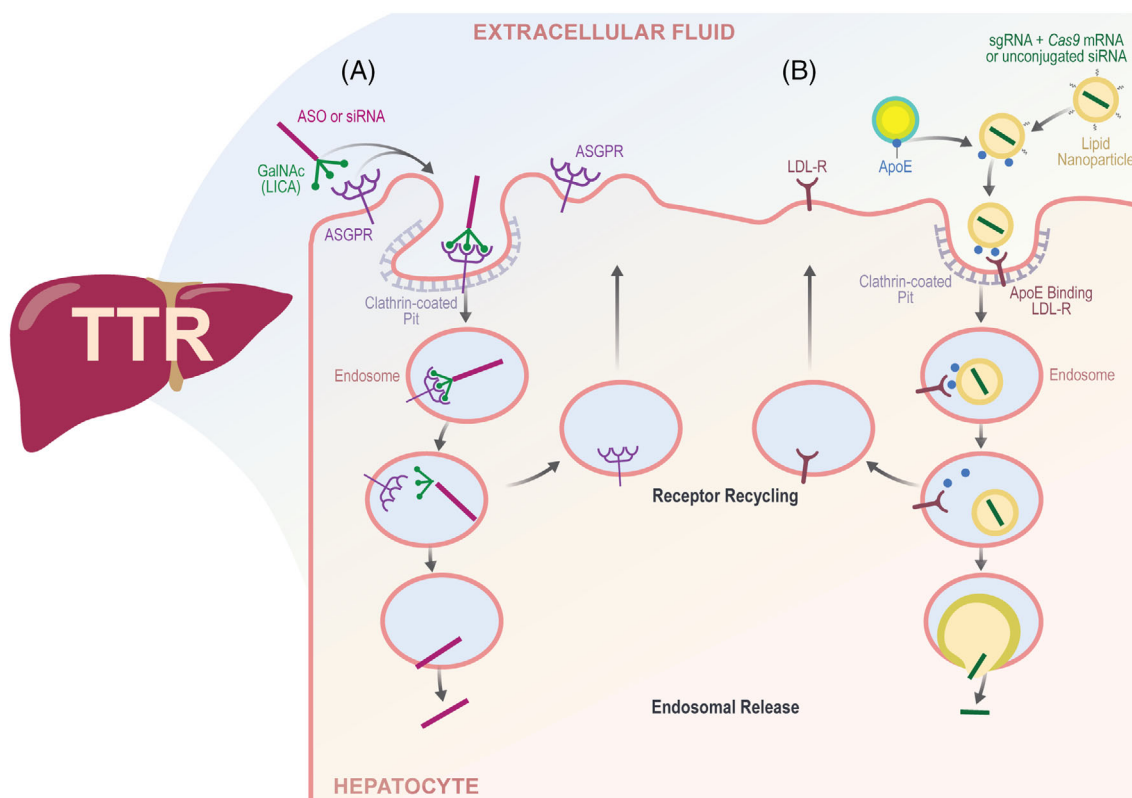
NTLA-2001 is an investigational, clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated (Cas) endonuclease tool that is intended to edit the DNA sequence in hepatocytes

responsible for *TTR* expression. It consists of a single guide RNA (sgRNA) and human-codon optimized *Cas9* mRNA sequence from *Streptococcus pyogenes* (which is translated into *Cas9* protein once inside the cell) encapsulated in a lipid nanoparticle.<sup>31</sup> The sgRNA has two functions: recognition of the target DNA and binding to the *Cas9* endonuclease (Figure 2C).<sup>31,64,65</sup> *Cas9* is responsible for the double-strand break that allows the desired editing outcome—which in this case is non-homologous end-joining (NHEJ) of the cleaved DNA strands. NHEJ is prone to generating insertion and deletion errors in the re-ligated DNA strands,<sup>31,65</sup> which effectively decreases functional *TTR* mRNA levels and subsequent production of *TTR*.

Published interim results from a phase 1, open-label study of six patients with ATTRv-PN showed that administration of NTLA-2001 was associated with a dose-dependent reduction in serum *TTR* levels for up to 28 days (Table 1).<sup>31</sup> NTLA-2001 is in trial for the treatment of both ATTRv-PN and all forms of ATTR-CM. Patients received glucocorticoid and histamine receptor type 1 and type 2 blockade before infusion to minimize allergic reactions to the lipid nanoparticle coat of the therapeutic.<sup>31</sup> With successful cleavage and subsequent knock-down of *TTR* DNA, this CRISPR-Cas9-based technology has the potential to become a one-time therapeutic strategy in patients with ATTR.<sup>31</sup>

## 4 | TARGETED HEPATIC DELIVERY OF RNA SILENCERS AND GENE EDITING TOOLS TO TREAT ATTR

There are currently two strategies used to enhance the delivery of RNA silencers and gene editing tools to hepatocytes. ASOs and siRNAs can be directly conjugated to a GalNAc ligand to support receptor-mediated uptake by the ASGPR. Alternatively, gene editing tools and siRNA can be encapsulated in hepatic-targeted LNP to



**FIGURE 3** Cellular Internalization and Release of GalNAc-conjugated molecules (A) vs. LNP-packaged molecules (B). Panel A: GalNAc-conjugated molecules include ASO or siRNA; oligonucleotide indicated by the pink bar. The GalNAc-oligonucleotide binds to asialoglycoprotein receptor (ASGPR) on the hepatocyte surface. Following endosome internalization, the GalNAc-oligonucleotide dissociates from ASGPR, allowing ASGPR to be recycled back to the hepatocyte surface. GalNAc is cleaved from the ASO or siRNA after internalization, and the active oligonucleotide is released into the cytosol. Panel B: LNP-packaged molecules include CRISPR-Cas9 therapies (ie, sgRNA + Cas9 mRNA) or unconjugated siRNA; nucleic acid cargo indicated by the green bar. After LNP administration, polyethylene glycol (PEG) lipids (indicated by black coils) dissociate and apolipoprotein E (ApoE) binds. The resulting LNP is delivered into liver sinusoids and is endocytosed into hepatocytes through the binding of low-density lipoprotein receptors (LDL-R). The LNP then fuses with the endosomal membrane, releasing the nucleic acid cargo into the cytosol.

support low-density lipoprotein (LDL) receptor-mediated uptake (Figure 3).

#### 4.1 | GalNAc conjugated ASO and siRNA

GalNAc conjugation to oligonucleotides is designed to support selective delivery of drugs to hepatocytes (Figure 3A). Typically, three GalNAc residues—referred to as a triantennary or trivalent cluster—are conjugated to the 5' end of the ASO or the 3' end of the siRNA passenger strand. Three residues are often used because triantennary GalNAc has high affinity and specificity for ASGPR, which most often exists in a trimer configuration.<sup>52,66–68</sup> ASGPR is primarily and highly expressed on hepatocytes, with approximately 500 000 receptors per cell, of which 5%–10% are present on the cell surface at any time. ASGPR clears serum glycoproteins via clathrin-mediated endocytosis and therefore has a high rate of uptake and rapid internalization and recycling (~15 min).<sup>52,57</sup> Upon binding to the ASGPR, the GalNAc-conjugated oligonucleotide is taken into the hepatocyte via endocytosis. Following

endosome internalization, a drop in pH leads to the dissociation of the GalNAc-oligonucleotide from ASGPR, allowing ASGPR to be recycled back to the hepatocyte surface.<sup>57</sup> GalNAc is rapidly cleaved from the ASO or siRNA after internalization by endosomal glycosidases, releasing the active oligonucleotide (Figure 3A).<sup>57,69</sup> Oligonucleotide functionality requires release from the endosome after internalization. It is believed that a small fraction of internalized oligonucleotides, likely <1%, are transported into the cytoplasm from endosomes.<sup>57,66</sup> Though limited numbers of oligonucleotides are released from endosomes, once within the cell, ASOs and siRNAs induce robust and sustained effects.

GalNAc conjugation to ASOs improves potency up to 30-fold in humans compared with unconjugated ASOs.<sup>30,70</sup> This allows for lower doses and less frequent dosing, with the potential for an improved safety profile.<sup>40,52</sup> In the NEURO-TTRtransform and the CARDIO-TTRtransform studies, 45 mg of the GalNAc conjugated ASO, eplontersen, is subcutaneously administered every 4 weeks either in the clinic or at home with an option for self-administration, while the unconjugated ASO with the same sequence (inotersen), requires 284 mg dosing once weekly to produce a similar level TTR protein reduction.<sup>47–49</sup>

siRNAs conjugated to GalNAc may be administered less frequently, subcutaneously, and with no pre-medication required (eg, corticosteroids), in contrast to LNP-encapsulated siRNA that commonly require intravenous administration.<sup>34,36,67,71</sup> The GalNAc conjugated siRNA vutrisiran is administered by a health care professional every 3 months in the HELIOS-A and HELIOS-B trials,<sup>62,63</sup> while the unconjugated, LNP formulated siRNA patisiran is administered every 3 weeks by IV infusion.<sup>56</sup>

## 4.2 | Hepatic targeted lipid nanoparticles

LNPs are both biocompatible and biodegradable and have been demonstrated to support nucleic acid delivery, including delivery of ATTR therapies to the liver.<sup>72-74</sup> After intravenous LNP administration, polyethylene glycol (PEG) lipids dissociate from the nanoparticle surface, allowing for apolipoprotein E (ApoE) binding (Figure 3B). The resulting opsonized LNP (ie, with bound ApoE) is delivered directly into liver sinusoids via systemic circulation, where it passes through endothelial fenestrae to hepatocytes. These LNPs are endocytosed into hepatocytes by binding to LDL receptors, which are primarily located on hepatocyte cell membranes. Within the acidic environment of the endosome, the LNP becomes positively charged and fuses with the negatively charged endosomal membrane so that the nucleic acid cargo can be released into the cytosol.

LNP technologies are currently used to deliver two ATTR gene silencers—an approved siRNA (patisiran) and a CRISPR gene-editing tool currently in development (NTLA-2001).<sup>31,73</sup> Both are administered intravenously and require premedication with a corticosteroid and antihistamine to mitigate potential infusion-related reactions.<sup>31,32</sup>

## 5 | ADVANTAGES AND CHALLENGES OF HEPATIC TARGETED RNA SILENCERS AND GENE EDITING TOOLS TO TREAT ATTR

The RNA silencing agents in clinical development that employ receptor-mediated uptake by hepatocytes have an extended pharmacological effect in comparison to the unconjugated silencers,<sup>40,51</sup> while gene editing tools could potentially require a single dose.<sup>31</sup> Less frequent dosing afforded by these hepatic targeted drugs may improve patient burden and compliance. In addition, the GalNAc conjugated ASOs improve potency with the potential for an improved safety profile.<sup>40</sup> Recent and upcoming readouts from the phase 3 clinical trials will be important to assess the efficacy and safety of these drugs for treating patients with all forms of ATTR amyloidosis.

While most TTR is produced and secreted by the liver, CNS and ocular manifestations of ATTR are thought to be due to amyloid deposition of non-hepatically-synthesized TTR that begins decades before the onset of symptoms.<sup>75-77</sup> CNS complications have been increasingly noted in patients following liver transplantation, suggesting that long-term survival may lead to amyloid deposits from CNS-synthesized mutant TTR—a complication not commonly reported in

the natural history of the disease.<sup>77</sup> CNS deposition following siRNA treatment has also been reported, such as a recently published case of leptomeningeal disease secondary to amyloidosis.<sup>78</sup> Additionally, long-term risk of ocular manifestations has been noted after liver transplantation and ATTR stabilization, due to intraocular production and deposition of mutant TTR in patients with prolonged survival.<sup>76,79</sup> These tissue compartments, however, are protected by the blood-brain barrier and inaccessible to current RNA silencers and gene editors administered by parenteral routes of delivery.<sup>80</sup> Access to these regions could potentially be addressed by intrathecal and/or intravitreal injection<sup>81,82</sup> or advancements in delivery across the blood-brain barrier.<sup>83-85</sup>

## 6 | CONCLUSION

Transthyretin-mediated amyloidosis is a progressive disease with various clinical manifestations due to TTR aggregation, deposition, and amyloid formation. Receptor-targeted ligands (eg, GalNAc) and nanoparticle encapsulation (eg, LNPs) are technologies to deliver ASOs, siRNAs, and gene editing tools to hepatocytes, the primary location of TTR synthesis. Our understanding of the long-term effects of hepatically targeted treatments on ATTR phenotypes—including the potential for CNS and ocular amyloidosis—will continue to grow as more patients receive treatment and RNA silencers and gene editing tools continue to improve. In the meantime, treatments that can suppress TTR protein synthesis remain a key strategy in improving survival and quality of life in patients with ATTR.

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