RNA-targeting and gene editing therapies for transthyretin amyloidosis

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Abstract | Transthyretin (TTR) is a tetrameric protein synthesized mostly by the liver and secreted into the plasma. TTR molecules can misfold and form amyloid fibrils in the heart and peripheral nerves, either as a result of gene variants in *TTR* or as an ageing-related phenomenon, which can lead to amyloid TTR (ATTR) amyloidosis. Some of the proposed strategies to treat ATTR amyloidosis include blocking TTR synthesis in the liver, stabilizing TTR tetramers or disrupting TTR fibrils. Small interfering RNA (siRNA) or antisense oligonucleotide (ASO) technologies have been shown to be highly effective for the blockade of *TTR* expression in the liver in humans. The siRNA patisiran and the ASO inotersen have been approved for the treatment of patients with ATTR variant polyneuropathy, regardless of the presence and severity of ATTR cardiomyopathy. Preliminary data show that therapy with patisiran improves the cardiac phenotype rather than only inducing disease stabilization in patients with ATTR variant polyneuropathy and concomitant ATTR cardiomyopathy, and this drug is being evaluated in a phase III clinical trial in patients with ATTR cardiomyopathy. Furthermore, ongoing phase III clinical trials will evaluate another siRNA, vutrisiran, and a novel ASO formulation, eplontersen, in patients with ATTR variant polyneuropathy or ATTR cardiomyopathy. In this Review, we discuss these approaches for *TTR* silencing in the treatment of ATTR amyloidosis as well as the latest strategy of genome editing with CRISPR–Cas9 to reduce *TTR* gene expression.

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Amyloidosis is a disorder characterized by the extracellular accumulation of misfolded proteins as insoluble fibrils, which cause tissue damage and organ dysfunction. The different forms of amyloidosis are classified according to the amyloidogenic precursor, which influences the patterns of organ deposition, natural history and the therapeutic approach[1]. Currently, 36 forms of amyloidosis are recognized, classified according to the type of protein accumulating in tissues[1]. Amyloid transthyretin (ATTR) amyloidosis is one of the most common forms of amyloidosis[2], caused by tissue deposition of full-length and fragmented monomers of transthyretin (TTR).

TTR is a homotetrameric protein that, in humans, is synthesized mainly in the liver and choroid plexus, from which it is secreted into the plasma and the cerebrospinal fluid, respectively[2]. In the plasma, TTR can bind to and transport the thyroid hormone thyroxine (T4), accounting for approximately 15% of the bound T4 pool; other carriers of T4 in the plasma include thyroxine-binding globulin (the major carrier) and albumin[2]. By contrast, in the cerebrospinal fluid, TTR is the major T4 carrier, transporting 80% of the hormone[2]. In addition, TTR also mediates the transport of vitamin A by associating with retinol-binding protein 4 (RBP4), which is the main carrier of vitamin A[2]. In agarose gel electrophoresis, TTR migrates in front of the albumin band, which is why TTR was formerly known as pre-albumin. Normal plasma concentrations of TTR vary between 20 mg/dl and 40 mg/dl (REF.[3]).

The *TTR* gene is located on chromosome 18q12.1 and encodes a 55-kDa tetramer with four identical monomers of 127 amino acids, each forming a β-sandwich with one small α-helix and eight β-strands[3]. During the 1990s, Kelly et al. demonstrated that dissociation of tetrameric TTR into monomers was followed by a rapid misfolding and misassembling of the monomers into aggregates, at least in vitro and in non-physiological conditions (low pH)[4,5]. A highly specific cleavage of the amyloid precursor by serine proteases might be particularly important to prime TTR fibrillogenesis under physiological conditions[6]. Among the >130 *TTR* variants identified[7], the vast majority are pathogenic and favour tetramer dissociation[8], whereas a few benign

Key points

- Transthyretin (TTR) is a tetrametric protein synthesized mainly by the liver that can misfold and deposit as amyloid fibrils, predominantly in peripheral nerves and the heart, which can result in amyloid TTR (ATTR) amyloidosis.
- Therapeutic options for ATTR amyloidosis include pharmacological agents that inhibit hepatic synthesis of TTR, stabilize the tetramer or disrupt the amyloid fibrils.
- The small interfering RNA (siRNA) patisiran and the antisense oligonucleotide (ASO) inotersen block liver *TTR* expression and have been approved for the treatment of variant ATTR polyneuropathy (ATTRv-PN).
- Phase III trials are ongoing on patisiran for the treatment of ATTR cardiomyopathy (ATTR-CM) and the siRNA vutrisiran for hereditary ATTRv-PN or ATTR-CM.
- A novel ASO formulation, eplontersen, is being evaluated in phase III trials in patients with ATTRv-PN or ATTR-CM.
- A genome editing strategy using CRISPR–Cas9 to silence the *TTR* gene is being investigated in a phase I trial.

TTR variants increase tetramer stability and are thereby protective[9].

Cardiac involvement is the main feature of wild-type ATTR (ATTRwt) amyloidosis, which is often associated with bilateral carpal tunnel syndrome and spinal canal stenosis[10] (BOX 1). The clinical presentation of variant ATTR (ATTRv) amyloidosis ranges from exclusive polyneuropathy (ATTRv-PN) to isolated cardiomyopathy (ATTR-CM), with a wide spectrum of mixed phenotypes[11] (BOX 2). The *TTR* variant Val30Met (p.Val50Met) accounts for the majority of ATTRv-PN cases[12]. Early-onset ATTRv-Val30Met amyloidosis slowly progresses from mild walking disturbances (stage 1) to being wheelchair-bound or bedridden (stage 3), until death occurring after a median of ~12 years from disease onset[11]. Late-onset ATTRv-Val30Met amyloidosis and ATTRv amyloidosis caused by other *TTR* variants usually have a more aggressive course, sometimes manifesting with severe gait unsteadiness and with patients showing a median survival of ~7 years[13,14]. ATTR-CM is a progressive disorder affecting patient outcomes and is associated with a median survival of 35 months in patients receiving placebo in the ATTR-ACT trial[15].

Orthotopic liver transplantation was first proposed in 1990 as a potentially curative treatment for ATTRv-PN[16]. Until 2011, orthotopic liver transplantation and combined liver–heart transplantation constituted the only available disease-modifying treatments for ATTRv amyloidosis[17]. Nonetheless, tissue TTR deposition can continue even after liver transplantation, probably because ATTRv deposits act as seeds for circulating wild-type TTR[18,19]. Over the past decade, different molecules targeting specific steps of the amyloidogenic cascade have been evaluated as possible drugs to modify patient outcomes[20] (FIG. 1; TABLE 1). Tetramer stabilizers prevent monomer misfolding and deposition by binding to the T4-binding site on TTR (for example, tafamidis and diflunisal) or by mimicking the structural influence of the super-stabilizing *TTR* variant T119M (for example, acoramidis)[20,21]. In a phase III clinical trial in patients with stage 1 early-onset ATTRv-Val30Met amyloidosis, tafamidis slowed the progression of neuropathy compared with placebo[21]. However, other open-label trials investigating the role of tafamidis in patients with late-onset ATTRv-Val30Met amyloidosis or ATTRv-PN caused by other *TTR* variants have provided neutral results[22–24]. On the basis of this conflicting evidence, in 2011, the EMA approved the use of tafamidis to treat patients with stage 1 ATTRv-PN, whereas the FDA rejected this indication. The phase III trial ATTR-ACT showed that patients with ATTR-CM (either variant or wild type) receiving tafamidis for 30 months had a lower risk of all-cause death or cardiovascular-related hospitalizations than patients receiving placebo[25]. Following these results, both the FDA (in 2019) and the EMA (in 2020) approved the use of tafamidis for the treatment of ATTR-CM.

Therapies inhibiting TTR production block the first step of the cascade and are well suited to influence the natural history of the disease. Small interfering RNA (siRNA) or antisense oligonucleotide (ASO) technologies can inhibit *TTR* expression in the liver with high efficiency. The siRNA patisiran and the ASO inotersen have been approved for the treatment of patients with stage 1 or stage 2 ATTRv-PN, regardless of the presence and severity of ATTR-CM[20]. Preliminary data have shown that patisiran improves the cardiac phenotype, rather than merely stabilizing disease, in patients with ATTRv-PN who have concomitant cardiac involvement[26]. This drug is currently being evaluated in a phase III trial in patients with predominant ATTR-CM[27]. In addition, vutrisiran is being investigated in phase III trials in patients with ATTRv-PN (HELIOS-A)[28] or ATTR-CM (HELIOS-B)[29]. Additionally, a novel formulation of ASO (eplontersen) showed promising preliminary safety and efficacy results in a phase I trial[30], and is being studied in phase III trials in patients with ATTRv-PN[31] or ATTR-CM[32]. Finally, an intriguing strategy of *TTR* gene editing for the treatment of ATTR amyloidosis has emerged[33]. In this Review, we provide an overview and an update on the approaches to knock down circulating *TTR* for the treatment of ATTR amyloidosis.

Small interfering RNAs

siRNAs are 20–25 nucleotide-long, double-stranded RNA molecules that can bind to complementary mRNA molecules and cause their degradation, thereby modulating gene expression[34,35]. siRNAs must avoid uptake by phagocytes, degradation by circulating nucleases and renal filtration, and must then be internalized in a cell-specific manner to be released into the cytoplasm of target cells[36,37] (FIG. 1). Several strategies have been developed to achieve these goals, including chemical modifications of specific nucleotides, ligand binding or the use of polymer-based or lipid-based delivery systems[38–40]. For example, conjugation with *N*-acetylgalactosamine (GalNAc) promotes the interaction with the asialoglycoprotein receptor, which is highly expressed by hepatocytes[37]. Numerous preclinical and clinical studies are ongoing to assess siRNA efficacy in disorders ranging from haematological diseases (haemophilia A and haemophilia B), cancer (pancreatic cancer and metastatic solid tumours), and hepatic (hepatitis B), eye (keratoconjunctivitis sicca and glaucoma) and kidney (primary hyperoxaluria) diseases[41–49], to cardiovascular risk factors such as hypertension and dyslipidaemias[50–53].

ATTR amyloidosis is a model of the scientific progress in the drug development of siRNA-based therapies because the siRNA patisiran has already entered the clinical testing stage and other siRNAs are being evaluated in phase II and III trials.

Patisiran

Formulation, mechanism of action, pharmacokinetics and drug interactions. Patisiran is a siRNA encapsulated into lipid nanoparticles (60–100 nm in diameter), which protect the RNA molecule from degradation by circulatory endonucleases and exonucleases and facilitate delivery to the liver. In serum, these lipid nanoparticles are coated by apolipoprotein E, which in turn binds to the LDL receptor on the hepatocyte membrane. In the cytoplasm, the RNA duplex unwinds and the antisense strand specifically binds to a genetically conserved sequence in the 3′ untranslated region (UTR) of *TTR* mRNA (either wild type or variant), blocking TTR protein synthesis[36].

At the recommended dosing regimen of 0.3 mg/kg every 3 weeks, a steady-state level of circulating patisiran is reached by 24 weeks[54]. Patisiran is metabolized into nucleotides of various lengths and <1% is excreted unchanged in the urine. The elimination half-life is 3 ± 2 days. Chronic kidney disease (down to an estimated glomerular filtration rate of 30 ml/min/1.73 m^2) and mild hepatic impairment have no substantial effect on drug pharmacokinetics. Patisiran does not inhibit or induce the activation of cytochrome P450 enzymes or transporters, and its pharmacokinetics are not influenced by concomitant use of strong or moderate inducers or inhibitors of cytochrome P450 3A[54].

Phase I and II clinical trials. Following a phase I trial[55], Suhr et al. conducted a phase II study including 29 adults with ATTRv amyloidosis who received two intravenous infusions of patisiran at 0.01, 0.05, 0.15 or 0.3 mg/kg every 4 weeks, or 0.3 mg/kg every 3 weeks[56]. After two doses of 0.3 mg/kg every 3 weeks, the mean reduction in serum TTR levels from baseline exceeded 85%, with a maximum of 96%[56]. In a 24-month, phase II, open-label extension (OLE) study[57] in 27 patients with ATTR amyloidosis, 11 of whom had cardiac disease, patisiran therapy (0.3 mg/kg every 3 weeks) was associated with halting of polyneuropathy progression or improvement in polyneuropathy scores compared with baseline. Motor function, autonomic symptoms, disease stage and quality of life remained stable during the 24-month study period. No significant changes were observed in echocardiographic measures or cardiac biomarkers[57].

Phase III clinical trials. The APOLLO trial[58] was a randomized, double-blind, placebo-controlled, phase III study enrolling 225 patients with ATTRv amyloidosis randomly assigned in a 2:1 ratio to patisiran 0.3 mg/kg or placebo every 3 weeks (TABLE 1). At 18 months, the modified Neuropathy Impairment Score + 7 (mNIS + 7) decreased by 6 ± 2 in the patisiran group compared with an increase by 28 ± 3 in the placebo group ($P < 0.001$); 56% of the patients receiving patisiran had an improvement in mNIS + 7 compared with 4% of patients receiving placebo. Significant between-group differences were observed across all secondary end points, demonstrating that patisiran treatment can lead to substantial improvements in neuropathy scores, quality of life, walking parameters and modified BMI[58].

A global OLE study[59] is evaluating the usual dose regimen in 137 patients treated with patisiran and 49 patients treated with placebo from the APOLLO trial, and 25 patients treated with patisiran from the phase II OLE study. In the 12-month interim analysis, patients treated with patisiran had a sustained improvement in mNIS + 7 (REF.[59]).

A prespecified subgroup analysis of the APOLLO trial evaluated 126 patients (56%) with a baseline left ventricular (LV) wall thickness ≥13 mm and no history of hypertension or aortic valve disease[26]. After 18 months,

Box 1 | **ATTR cardiomyopathy**

Epidemiology

Among Americans of European descent, amyloid transthyretin (ATTR) variant (ATTRv) amyloidosis has an estimated incidence of 0.4 per million people per year, but this condition is believed to be more common among people with African ancestry and in specific geographical areas such as northern Portugal or some regions of West Africa[90]. Conversely, wild-type ATTR (ATTRwt) amyloidosis is a sporadic disorder with no specific biomarkers for its diagnosis, most often affecting elderly men[90]. An autopsy study reported amyloid deposition in 25% of patients aged >85 years[91], although the clinical relevance of amyloid deposits per se is unclear, and only severe and widespread amyloid accumulation is likely to produce disease manifestations. When systematically screened, ATTR cardiomyopathy (ATTR-CM) was diagnosed in 13% of patients referred for transcatheter aortic valve implantation[92] and in 13% of patients with heart failure with preserved ejection fraction[93], suggesting that this condition is largely underdiagnosed.

Clinical manifestation

ATTR-CM should be screened in patients with symptomatic heart failure, syncope or bradyarrhythmia, with imaging findings suggestive of cardiac amyloidosis. In endemic areas, the diagnostic process can be facilitated by family history, the prototypic clinical presentation and the detection of the *TTR* variant. The presence of cardiac abnormalities, including intracardiac conduction disorders, symptoms of dysautonomia and carpal tunnel syndrome, should suggest the diagnosis of ATTR amyloidosis. Cardiac involvement can manifest with left ventricular (LV) pseudohypertrophy and/or overt heart failure with preserved ejection fraction, possibly accompanied by conduction disturbances or arrhythmias. ATTR-CM should be differentiated from LV hypertrophy secondary to pressure or volume overload as well as from primary hypertrophy caused by variants in genes encoding sarcomere proteins, other infiltrative conditions, or other aetiologies[94]. ATTR-CM should be considered in all patients who have LV wall thickening, especially when QRS voltages are normal or low and the left ventricle is not enlarged[95]. The likelihood of diagnosis increases when cardiac biomarker levels (troponins and/or natriuretic peptides) are increased and symptoms or history of peripheral or autonomic neuropathy are present[95].

Diagnosis

Diagnosis can be made either through the demonstration of ATTR presence on a myocardial biopsy sample or with the use of a non-invasive algorithm in patients with no evidence of a monoclonal protein, in whom bone scintigraphy with ^{99m}Tc-labelled radiotracers has a central role[69].

Treatment

Tafamidis is the only approved treatment for ATTR-CM. Tafamidis stabilizes TTR tetramers and inhibits tissue deposition of TTR monomers. In a phase III trial[25], patients receiving tafamidis for 30 months had a lower risk of all-cause death or cardiovascular-related hospitalizations than patients receiving placebo. The survival benefit became evident after 18 months of treatment initiation[25]. Heart failure management in ATTR-CM relies mostly on diuretics and mineralocorticoid receptor antagonists. The role of β-blockers, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers and digoxin is controversial, and calcium-channel blockers should be avoided. The indications to pacing and defibrillator therapy and atrial fibrillation ablation remain to be clarified.

Box 2 | **ATTRv polyneuropathy**

Definition

Amyloid transthyretin variant (ATTRv) amyloidosis with polyneuropathy (ATTRv-PN) is a disease affecting the somatic and autonomic peripheral nervous system owing to variants in the *TTR* gene inherited with an autosomal dominant trait. Familial amyloid polyneuropathy refers to all forms of inherited amyloid polyneuropathies, with ATTRv-PN being by far the most common[12,96].

Epidemiology

ATTRv amyloidosis has been reported in 36 countries, with the biggest historical clusters (endemic areas) in Portugal, Japan and Sweden[97]. The Val30Met (p.Val50Met) *TTR* variant accounts for the majority of ATTRv-PN cases both in endemic and non-endemic areas, although other specific variants can be prevalent in certain regions, such as Thr60Ala in Northern Ireland, Glu89Gln in Bulgaria, Ser50Arg in Mexico, Phe64Leu in Sicily, Ser77Tyr and Ser77Phe in France, and Ala97Ser in Taiwan[12]. Val122Ile is the most common variant in the USA and is primarily observed in individuals of African descent[90]. In Portugal, the prevalence of ATTRv amyloidosis in 2016 was estimated to be 22.93 per 100,000 adults (45.8% male; mean age 52.3 ± 15.4 years)[98]. On the basis of the estimated prevalence in 10 'core' countries (Bulgaria, Cyprus, France, Germany, Italy, Japan, the Netherlands, Portugal, Sweden and Turkey), a study from 2018 calculated a global prevalence of ATTRv amyloidosis of 10,186 (range 5,526–38,468)[99].

Clinical manifestation

ATTRv amyloidosis is classified into neurological, cardiac or mixed phenotypes according to the initial presentation, which depends on the specific *TTR* variant[12]. ATTRv-PN is the most common initial manifestation but a mixed phenotype often ensues with increasing age. Indeed, cardiac involvement has been observed in 56–63% of patients with ATTRv-PN[58,73]. ATTRv-PN manifests in two main forms: early-onset (age <50 years) or late-onset (age ≥50 years) ATTRv-Val30Met amyloidosis. ATTRv-PN caused by other *TTR* variants usually resembles the late-onset ATTRv-Val30Met amyloidosis phenotype independently of age[12,97]. Early-onset ATTRv-Val30Met amyloidosis is characterized by a length-dependent, small-fibre polyneuropathy with a predominant loss of thermal and pain sensory fibres. Symptoms include lower-limb paranesthesia and altered pain and temperature sensation starting from the feet and progressing proximally, sometimes associated with plantar ulcers. Conversely, late-onset ATTRv-Val30Met amyloidosis can present with either exclusive lower-limb, upper-limb or all four-limb involvement, always in a disto-proximal fashion; carpal tunnel syndrome occurs in 23–63% of patients[12,97]. Autonomic involvement is common in both early-onset and late-onset forms, manifesting with variable symptoms including gastrointestinal disorders, dysuria, erectile dysfunction and orthostatic hypotension[12,97]. Early-onset ATTRv-Val30Met amyloidosis slowly progresses from mild walking disturbances (stage 1) to being wheelchair-bound or bedridden (stage 3) until death occurring after a median of ~12 years from disease onset. Late-onset ATTRv-Val30Met amyloidosis and ATTRv owing to other *TTR* variants usually have a more aggressive course, sometimes manifesting with severe gait unsteadiness, with patients showing a median survival of ~7 years[13,14].

Diagnosis

The diagnosis of polyneuropathy is made through clinical assessment through specific scores, combined with nerve conduction studies of sensorimotor function and tests aimed to evaluate autonomic function (such as measurement of heart rate variability, head-up tilt-test for orthostatic hypotension and electrochemical skin conductance for sudomotor function). Definite diagnosis of ATTRv-PN is made through a biopsy analysis documenting amyloid deposits and through genetic testing to detect amyloidogenic *TTR* variants. In endemic areas, carrier status is often known before disease onset thanks to family screening; therefore, ATTRv-PN is diagnosed through periodic monitoring of clinical status[12,97].

Treatment

Until 2011, the only approved disease-modifying treatment for ATTRv-PN was liver transplantation[17]. Nonetheless, deposition of wild-type TTR can progress over already formed ATTR deposits even after liver transplantation[19]. Symptomatic therapy includes physical rehabilitation, painkillers and treatments of autonomic dysfunction (diarrhoea, erectile dysfunction and orthostatic hypotension)[12].

Tafamidis, a TTR stabilizer, slowed neuropathic progression compared with placebo in a phase III trial in patients with stage 1 early-onset ATTRv-Val30Met amyloidosis[21]. By contrast, open-label studies of tafamidis in patients with late-onset ATTRv-Val30Met amyloidosis or ATTRv-PN owing to other *TTR* variants have had inconclusive results[22–24]. On the basis of these data, the EMA approved, in 2011, the use of tafamidis for the treatment of patients with stage 1 ATTRv-PN, whereas the FDA rejected its use in this clinical setting because of insufficient evidence for its efficacy. After the positive results of the phase III trials APOLLO[58] and NEURO-TTR[73], patisiran (a small interfering RNA) and inotersen (an antisense oligonucleotide) were approved in 2018 by both the EMA and FDA for the treatment of adult patients with stage 1 or stage 2 ATTRv-PN, representing nowadays the mainstay of therapy in this clinical setting.

patisiran therapy seemed to reduce cardiac dysfunction by lessening the amyloid burden[26]. Indeed, global longitudinal strain decreased in the patisiran group compared with placebo (least-squares mean difference ± standard error −1.4 ± 0.6%, $P = 0.015$) and cardiac output increased (0.38 ± 0.19 l/min, $P = 0.044$). Patients receiving patisiran had a slight but significant reduction in LV wall thickness compared with the placebo group (−0.9 ± 0.4 mm; $P = 0.017$)[26]. Furthermore, patients receiving patisiran had a 46% reduction in the rate of cardiac-related hospitalizations and all-cause death compared with those receiving placebo[26]. A study published in 2021 compared 16 patients with ATTRv amyloidosis receiving patisiran for 12 months (together with diflunisal in 12 of the patients) and 16 untreated patients with ATTRv amyloidosis[60]. The investigators reported a reduction in estimated extracellular volume by cardiac magnetic resonance (CMR) in patients receiving patisiran compared with untreated patients (mean difference −6.2%; $P = 0.001$) paralleled by a reduction in cardiac uptake of bone radiotracers, an increase in 6-min walking distance (6MWD) and a reduction in N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels. Nonetheless, no change in LV structure or function was observed by CMR[60], underscoring the need for further imaging studies demonstrating a regression of amyloid cardiomyopathy with patisiran therapy.

The APOLLO-B trial[27] was designed to investigate patisiran as a treatment for ATTR-CM. This trial is currently enrolling patients with ATTRv-CM or ATTRwt-CM with a history of heart failure (but current clinically stable), NT-proBNP levels ranging from 300 ng/l to 8,500 ng/l and a 6MWD of ≥150 m. Patients must also be naive from tafamidis treatment or show evidence of disease progression with tafamidis treatment. The primary outcome and first secondary outcome measures are changes in 6MWD and in Kansas City Cardiomyopathy Questionnaire Overall Summary Score (KCCQ-OS) over 12 months, respectively. Study results are expected in mid-2022 (REF.[27]).

Safety. In the phase II study, mild-to-moderate infusion-related reactions occurred in 10% of patients receiving patisiran[56]. No clinically significant changes in liver function tests, renal function or haematological parameters were observed, and no dose-limiting toxicities or deaths caused by adverse events were recorded[56]. In addition, no drug-related adverse events leading to treatment discontinuation were recorded during the 24-month phase II OLE study[59].

Most of the adverse events in the APOLLO trial were mild or moderate[58]. The most frequent adverse events were diarrhoea (37% in the patisiran group versus 38% in the placebo group), peripheral oedema (30% versus 22%), falls (17% versus 29%), nausea (15% versus 21%), infusion-related reactions (19% versus 9%), constipation (15% versus 17%) and urinary tract infection (13% versus 18%)[58]. The frequency of serious adverse events was 36% and 40% in the patisiran and placebo groups, respectively, and adverse events leading to discontinuation of the trial regimen occurred less often with patisiran than with placebo (5% versus 14%). The incidence of cardiac adverse events (28% in the patisiran group

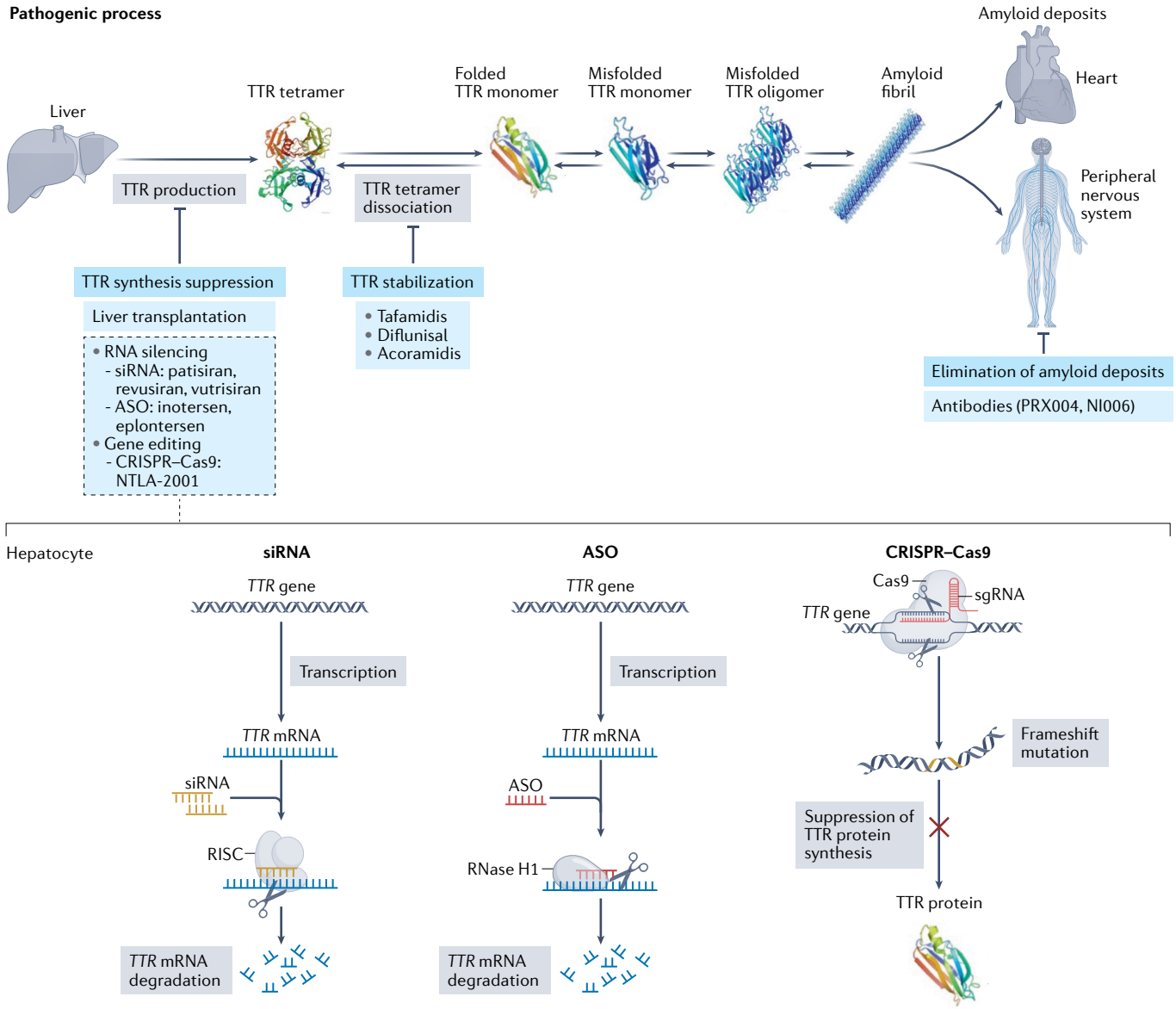

Fig. 1 | **RNA-targeting and gene editing strategies for the treatment of amyloid TTR amyloidosis.** Transthyretin (TTR) is a homotetrameric protein mainly synthesized in the liver that, under amyloidogenic conditions, can dissociate into aggregation-prone intermediates that self-assemble into amyloid fibrils. Over the past decade, different strategies targeting specific steps of the amyloidogenic cascade have been evaluated. Therapies inhibiting TTR production include liver transplantation, RNA-targeted gene silencing, such as small interfering RNAs (siRNAs) (patisiran, revusiran and vutrisiran) and antisense oligonucleotides (ASOs) (inotersen and eplontersen), and gene editing strategies such as CRISPR–Cas9 technologies (NTLA-2001). siRNAs and ASOs act by binding to complementary *TTR* mRNA molecules and causing their degradation or silencing. Gene editing with CRISPR–Cas9 induces a double-strand break in the *TTR* gene, ultimately preventing the production of the protein. Tetramer stabilizers, such as tafamidis and diflunisal, are small molecules that influence the rate-limiting step in the formation of amyloid fibrils, namely the dissociation of TTR tetramers into amyloidogenic monomers. Finally, anti-TTR antibodies have been shown to be effective in disrupting amyloid deposits[73]. RISC, RNA-induced silencing complex; sgRNA, single-guide RNA.

Table 1 | **Clinical trials of RNA-targeting and gene editing therapies for the treatment of ATTR amyloidosis**

Drug	Drug type	Study name (year); refs	Study design	Study population	Main efficacy results	Safety outcomes	Approved indications[a]
Patisiran	siRNA LNP	APOLLO (2018)[27,58]	Phase III, multicentre, randomized, double-blind, placebo-controlled; 2:1 randomization to IV patisiran (0.3 mg/kg) or placebo once every 3 weeks for 18 months	225 patients with ATTRv-PN (patisiran $n = 148$; placebo $n = 77$)	Patisiran significantly improved neuropathy scores, QOL, walking parameters, nutritional status and activities of daily living	Most adverse events were mild or moderate; similar frequency of serious adverse events in the patisiran and placebo groups; adverse events leading to drug discontinuation were less common with patisiran than with placebo	FDA: adults with ATTRv-PN EMA: adults with stage 1–2 ATTRv-PN
				126 (56%) patients had ATTRv-CM	Subgroup with cardiac disease: patients receiving patisiran had lower increase in LVEDV, lower decrease in cardiac output, and lower mean LV wall thickness, relative wall thickness, LV mass and NT-proBNP level at 18 months than patients receiving placebo		
		Adams et al. (2021)[59]	Multicentre, open-label extension, including patients from phase II studies and from APOLLO trial	211 patients with ATTRv-PN (patisiran $n = 162$; placebo $n = 49$)	At the interim 12-month follow-up, sustained improvements were observed in neuropathy-related scores with patisiran treatment		
Revusiran	GalNAc-siRNA	ENDEAVOUR (2020)[63]	Phase III, multicentre, randomized, double-blind, placebo-controlled; 2:1 randomization to SC revusiran (500 mg) or placebo daily for 5 days, then weekly for 18 months	206 patients with ATTRv-CM (revusiran $n = 140$; placebo $n = 66$)	NA (trial stopped)	Increased mortality with revusiran compared with placebo	Drug development discontinued
Vutrisiran	GalNAc-siRNA	HELIOS-A[28]	Phase III, multicentre, randomized, open-label; 3:1 randomization to SC vutrisiran (25 mg) once every 3 months or IV patisiran (0.3 mg/kg) once every 3 weeks, for 18 months	164 patients with ATTRv-PN (vutrisiran $n = 122$; patisiran $n = 42$)	At 9 months, vutrisiran significantly improved neuropathy scores, QOL and gait speed	No serious adverse events	Evaluation ongoing
		HELIOS-B[67]	Phase III, multicentre, randomized, double-blind, placebo-controlled; SC vutrisiran (25 mg) or placebo once every 3 months	Patients with ATTRv-CM or ATTRwt-CM	NA		
Inotersen	2′-MOE-modified ASO	NEURO-TTR (2018)[73]	Phase III, multicentre, randomized, double-blind, placebo-controlled; 2:1 randomization to weekly SC inotersen (300 mg) or placebo	172 patients with stage 1–2 ATTRv-PN (inotersen $n = 112$; placebo $n = 60$)	Inotersen modified the course of neuropathy and improved QOL independently of *TTR* variant type, disease stage or cardiac involvement status at baseline	Increased risk of glomerulonephritis and thrombocytopenia; adverse events leading to drug discontinuation more common with inotersen than placebo	FDA: adults with ATTRv-PN EMA: adults with stage 1–2 ATTRv-PN
				108 (63%) patients had ATTRv-CM	No differences in global longitudinal strain and other echocardiographic variables		
		NCT03702829 (2020)[72]	Phase I, single-centre; weekly SC inotersen (300 mg)	Patients with ATTR-CM	NA		

Table 1 (cont.) | **Clinical trials of RNA-targeting and gene editing therapies for the treatment of ATTR amyloidosis**

Drug	Drug type	Study name (year); refs	Study design	Study population	Main efficacy results	Safety outcomes	Approved indications[a]
Eplontersen	2′-MOE-modified, GalNAc3-conjugated ASO	Viney et al. (2021)[30]	Phase I, single-centre, randomized, placebo-controlled; 10:2 randomization (active versus placebo), one cohort receiving a single SC dose of eplontersen 120 mg and three cohorts receiving SC eplontersen 45 mg, 60 mg or 90 mg once every 4 weeks	Healthy volunteers ($n = 47$; 20 women, 27 men)	Eplontersen produced an overall reduction of approximately 90% in circulating TTR levels in the multiple-dose cohorts	No serious adverse events	Evaluation ongoing
		NCT04843020 (2021)[75]	Phase I, single-centre; monthly SC eplontersen (45 mg)	Patients with ATTR-CM	NA		
		CARDIO-TTRansform (2022)[77]	Phase III, multicentre, randomized, double-blind, placebo-controlled	Patients with ATTR-CM	NA		
NTLA-2001	Gene editing (LNP-based CRISPR–Cas9 with *TTR*-specific RNA and Spy Cas9 mRNA)	Gillmore et al. (2021)[33]	Phase I, multicentre, randomized, open-label, placebo-controlled; single IV dose of NTLA-2001: 0.1 mg/kg ($n = 3$) or 0.3 mg/kg ($n = 3$)	6 patients with ATTRv-PN	Interim results from the first two single-dose groups of the trial: NTLA-2001-mediated dose-dependent and sustained reductions in serum TTR protein concentration	No serious adverse events[b]	Evaluation ongoing

2′-MOE, 2′-*O*-methoxyethyl; ASO, antisense oligonucleotide; ATTR, amyloid transthyretin; ATTR-CM, transthyretin amyloidosis with cardiomyopathy; ATTRv-CM, variant transthyretin amyloidosis with cardiomyopathy; ATTRv-PN, variant transthyretin amyloidosis with polyneuropathy; ATTRwt-CM, wild-type transthyretin amyloidosis with cardiomyopathy; GalNAc, *N*-acetylgalactosamine; IV, intravenous; LNP, lipid nanoparticle; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; NA, not applicable; NT-proBNP, N-terminal pro-B-type natriuretic peptide; QOL, quality of life; siRNA, small interfering RNA; SC, subcutaneous; TTR, transthyretin. [a]FDA and EMA, by December 2021. [b]Follow-up limited to 28 days after injection.

versus 36% in the placebo group), cardiac serious adverse events (14% versus 13%) and heart failure (9% versus 10%) was similar in the two groups[58]. The incidence of cardiac arrhythmias was lower with patisiran (19%) than with placebo (29%)[58]. In the 12-month global OLE study[59], 39% of patients reported serious adverse events but only 1% of patients had serious adverse events that were considered related to treatment: one patient had abdominal discomfort and one patient had two events associated with extravasation of the study drug.

Regulatory approval. Patisiran was approved in 2018 by the FDA and the EMA for the treatment of adult patients with stage 1 or stage 2 ATTRv-PN. For patients weighing <100 kg, the recommended dosage is 0.3 mg/kg once every 3 weeks. For patients weighing ≥100 kg, the recommended dosage is 30 mg once every 3 weeks. Given that patisiran can reduce serum vitamin A levels, a supplementation of approximately 2,500 IU of vitamin A per day is advised[54,61].

Revusiran

Revusiran is a siRNA directed against *TTR* mRNA, conjugated to a GalNAc ligand that targets it to the liver[62]. Revusiran was investigated in the phase II ENDEAVOUR trial[63], which was designed to assess 6MWD and serum TTR in 200 patients with ATTRv-CM. Patients were randomly assigned 2:1 to revusiran (subcutaneous administration of 500 mg daily for 5 days, then weekly for 18 months) or placebo (TABLE 1). The study was discontinued after 13% of patients receiving revusiran and 3% receiving placebo died during a median of 7 months[57]. Most patients died because of heart failure. These patients were usually aged ≥75 years and had more advanced heart failure at baseline compared with those who were alive throughout the experimental protocol[63].

Vutrisiran

Formulation, mechanism of action and pharmacokinetics. Vutrisiran (also known as ALN-TTRsc02) is another siRNA targeting *TTR* mRNA[64]. Vutrisiran is a GalNAc conjugate that uses an enhanced stabilization chemistry–GalNAc conjugate delivery platform[65], which enables subcutaneous administration with longer intervals compared with patisiran. In a phase I study[64], vutrisiran showed a rapid absorption after subcutaneous administration. The maximum serum concentration (C_{max}) was reached between 3 h and 5 h after administration and increased in a dose-proportional manner. The mean half-life was 4.2–7.5 h. The fraction of renal clearance to total clearance ranged from 15.5% to 27.5%,

which meant that renal excretion was a minor route of elimination for vutrisiran[64].

Phase I clinical trial. A phase I, randomized, single-blind, placebo-controlled study[64] included 80 healthy individuals who received a single dose (5, 25, 50, 100, 200 or 300 mg) of vutrisiran ($n = 60$) or placebo ($n = 20$). Vutrisiran treatment was safe and well tolerated (as explained below). The mean maximal reduction in plasma TTR levels was 57–97%, and nadir TTR levels were achieved by 50–90 days and maintained for approximately 90 days at doses of ≥25 mg. Plasma TTR levels began to recover in all dosage groups after day 90, with a slower rate of recovery in the higher dose groups[64].

Phase III clinical trials. The HELIOS-A trial[28,66] is an ongoing phase III, global, multicentre, randomized, open-label study that enrolled 164 patients with ATTRv-PN to assess the efficacy and safety of vutrisiran (TABLE 1). Study participants received a 25 mg dose of vutrisiran once every 3 months ($n = 122$) or the reference comparator patisiran ($n = 42$). Patients who had received any experimental drug within 30 days of dosing or previous TTR-lowering treatment were excluded but those receiving ongoing therapy with tafamidis were not. At 9 months, vutrisiran met the primary end point and all secondary end points[66]. Specifically, vutrisiran treatment resulted in a 2.24-point mean decrease (improvement) in mNIS + 7 score from baseline at 9 months compared with a 14.76-point mean increase (worsening) reported for the external placebo group (from the APOLLO trial; $n = 77$), resulting in a 17.0-point mean difference relative to placebo ($P < 0.001$)[66]. Improvement in mNIS + 7 with vutrisiran treatment was also consistently observed across all prespecified patient subgroups, including patients with cardiac disease. Vutrisiran also resulted in a 3.3-point mean decrease (improvement) in the Norfolk Quality of Life Questionnaire–Diabetic Neuropathy (Norfolk QOL-DN) score from baseline at 9 months compared with a 12.9-point mean increase (worsening) reported for the external placebo group, equating to a mean 16.2-point difference relative to placebo ($P < 0.001$)[66].

The ongoing HELIOS-B trial[67] is a phase III, randomized, double-blind, placebo-controlled, multicentre study exploring the efficacy and safety of vutrisiran in patients with ATTRv-CM or ATTRwt-CM (TABLE 1). Participants will receive subcutaneous injections of vutrisiran 25 mg once every 3 months. The primary outcome is a composite of all-cause mortality, cardiovascular hospitalizations and urgent hospital visits related to heart failure at 30–36 months. Secondary outcomes include change from baseline in 6MWD, KCCQ-OS, NT-proBNP levels, mean LV wall thickness and global longitudinal strain, and the composite of all-cause mortality and recurrent cardiovascular events at month 30 or from 30 months to 36 months. Patients with non-TTR cardiomyopathy or who had received previous TTR-lowering treatment are excluded from the trial[67]. The results from HELIOS-B are expected in early 2024 (REF.[68]).

Safety. In the phase I study, adverse events were reported in 46 (77%) patients in the vutrisiran group and 10 (50%) in the placebo group[64]. All adverse events were mild. The most frequent adverse events were nasopharyngitis (52% in the vutrisiran group versus 30% in the placebo group), headache (17% versus 0%), diarrhoea and nausea (8% versus 0%), and pain in the injection site (5% versus 5%). Transient elevations of alanine aminotransferase levels of <3 times the upper limit of normal were observed in some treated patients, mostly at vutrisiran doses of ≥100 mg. Therapy with vutrisiran did not elicit a clinically relevant antibody response[64]. In the HELIOS-A study, vutrisiran had an encouraging safety and tolerability profile compared with placebo at 9 months of dosing, and no drug-related discontinuations or deaths occurred[66].

Antisense oligonucleotides

ASOs are 16–20 base-pair, single-strand, synthetic oligonucleotides that can silence target mRNA sequences by a variety of mechanisms, mostly involving degradation mediated by the endogenous ribonuclease RNase H1 (REF.[69]) (FIG. 1). Several ASO-based drugs have already been approved by the FDA as disease-modifying therapies for several conditions, including cytomegalovirus retinitis, Duchenne muscular dystrophy, familial amyloid polyneuropathy, homozygous familial hypercholesterolaemia, neovascular age-related macular degeneration, sinusoidal obstruction syndrome and spinal muscular atrophy[69].

Inotersen

Formulation, mechanism of action and pharmacokinetics. Inotersen is a 2′-*O*-methoxyethyl-modified ASO inhibitor of *TTR* expression that works by binding the 3′ untranslated region of human *TTR* mRNA (both wild-type and variant *TTR*). After subcutaneous injection, inotersen is rapidly absorbed into the systemic circulation and circulates mostly (>94%) bound to plasma proteins[70]. The C_{max} is reached within 1.5–4 h after injection. By 24 h after injection, a >90% reduction from C_{max} is observed owing to rapid transfer of the drug from plasma to tissues; afterwards, a slow elimination occurs, with an estimated terminal half-life of approximately 1 month[70]. Inotersen is not metabolized by cytochrome P450 but is degraded by endonucleases to shorter inactive oligonucleotides, which are further cleaved. Both inotersen and its metabolites are excreted in the urine[70].

Phase I clinical trials. Inotersen was tested in a phase I, randomized, placebo-controlled, double-blind, dose-escalation study[71]. The multiple-dose trial lasted 4 weeks, with three doses being administered on alternative days during the first week and three other doses once weekly for the remaining 3 weeks. Patients in the multiple-dose cohort showed a dose-dependent reduction in circulating TTR levels, with mean percentage change from baseline to week 4 in serum TTR levels of −8%, −22%, −53%, −75% and −76% in the 50, 100, 200, 300 and 400 mg dose regimens, respectively[71]. TTR suppression was prolonged over time; indeed, the 300 mg and 400 mg cohorts showed a 30% reduction below baseline in mean

serum TTR levels at 10 weeks after the last inotersen dose. Circulating levels of RBP4 paralleled the reduction observed for TTR[71]. Vitamin A levels in serum also decreased over time after treatment with inotersen but no adverse events related to vitamin A deficiency were observed[71]. No serious adverse events were reported in any treatment regimen subgroups[71].

Phase II trial. An ongoing phase II, single-centre, open-label study is evaluating the efficacy and safety of inotersen 300 mg weekly for 24 months in 50 patients with ATTR-CM (either variant or wild type)[72]. Patients are undergoing echocardiographic assessment at baseline and at 12, 18 and 24 months, and CMR is performed (when feasible) at baseline and at 6, 12 and 24 months. The primary end point is a change in longitudinal LV strain from baseline. Results are expected in 2022.

Phase III clinical trial. The NEURO-TTR trial[73] was a double-blind, placebo-controlled, phase III study that enrolled 172 patients with stage 1 (ambulatory) or stage 2 (ambulatory with assistance) ATTRv amyloidosis with polyneuropathy, who were randomly assigned in a 2:1 ratio to receive subcutaneously administered inotersen (300 mg) or placebo once a week (TABLE 1). Of the enrolled patients, 139 (81%) completed the 15-month intervention period. The difference in the least-squares mean change from baseline to week 66 between inotersen and placebo favoured inotersen for both primary efficacy assessments: −19.7 points for mNIS + 7 ($P < 0.001$) and −11.7 points for the Norfolk QOL-DN score[73]. Notably, 37% of the patients receiving inotersen had an absolute improvement in mNIS + 7 compared with 19% in the placebo group, and 50% had an absolute increase in the Norfolk QOL-DN score compared with 27% in the placebo group. In the inotersen group, a steady-state reduction in circulating TTR levels was reached approximately 13 weeks after treatment initiation, resulting in a median TTR reduction of 79%[73]. The benefit of inotersen treatment seemed independent from the degree of TTR suppression. Indeed, the absolute or relative change from baseline in serum TTR levels did not correlate with the change from baseline in mNIS + 7 (REF.[73]). The difference in the primary efficacy measures between patients receiving inotersen and those receiving placebo were maintained across all prespecified subgroups (Val30Met *TTR* versus other variants, stage 1 versus stage 2 and previous treatment with tafamidis or diflunisal versus no previous treatment) as well as in patients with or without cardiac involvement. In both the whole study population and in the subgroup with cardiac disease, no differences in global longitudinal strain or other echocardiographic variables (wall thickness, LV mass, LV ejection fraction and lateral E/e′) were observed between the inotersen and placebo groups at the 15-month follow-up[73].

Safety. In the NEURO-TTR trial[73], adverse events were the main reason for drug discontinuation in the inotersen group (14% of patients). Injection site reactions, nausea, headache, fatigue, fever and thrombocytopenia occurred in at least 20% of patients treated with inotersen. All five deaths during the intervention period occurred in the inotersen group, two owing to cachexia, one to intestinal perforation, one to heart failure progression and, importantly, one caused by intracranial haemorrhage in a patient with severe thrombocytopenia[73]. In the inotersen group, 54% of patients had a platelet count of <140,000/mm^3, falling to <25,000/mm^3 in 3% of patients. The most likely mechanism of reduced platelet counts is immune-mediated thrombocytopenia because some patients recovered with treatment with glucocorticoids and had anti-platelet antibodies[73]. Frequent platelet monitoring is recommended both before starting inotersen therapy and during treatment[70]. Glomerulonephritis occurred in 3% of patients receiving inotersen[73], which is why frequent monitoring of renal function (before treatment initiation and during treatment) is recommended by FDA labelling[70]. All patients in the NEURO-TTR trial received vitamin A supplementation; accordingly, no clinical manifestations of vitamin A deficiency were reported[73].

Regulatory approval. Inotersen received regulatory approval in 2018 by the FDA[70] and the EMA[74] for the treatment of adult patients with stage 1 or stage 2 ATTRv-PN at a dosage of 284 mg weekly. Inotersen is contraindicated in patients with a platelet count of <100,000/mm^3, an estimated glomerular filtration rate of <45 ml/min/1.73 m^2 or a urine protein to creatinine ratio of ≥113 mg/mmol (1 g/g). Oral supplementation of approximately 3,000 IU of vitamin A daily is advised for all treated patients[70,74].

Eplontersen

Formulation, mechanism of action and pharmacokinetics. Eplontersen is an ASO with an identical sequence as inotersen, conjugated to a triantennary GalNAc (GalNAc3), which allows binding to the high-capacity asialoglycoprotein receptors expressed by hepatocytes[30]. After cell internalization, the GalNAc3 moiety is cut via an endocytic pathway to release a 'free ASO' inside the hepatocyte[30]. In human hepatocytes in vitro, eplontersen was approximately 50-fold more potent than inotersen in reducing *TTR* expression[30]. Similarly, in transgenic mice expressing the human Ile84Ser *TTR* variant, eplontersen induced a 28-fold more potent knockdown of *TTR* than inotersen, which translated into a 15-fold greater reduction in plasma TTR levels[30]. In humans, eplontersen rapidly distributes into the systemic circulation after subcutaneous administration, with a median time to C_{max} of 1–6 h. Similar to inotersen, the C_{max} of eplontersen declines in a biphasic fashion, with an estimated terminal half-life of 2–4 weeks[30].

Phase I and II clinical trials. In a randomized, placebo-controlled, phase I study evaluating eplontersen in healthy volunteers, 11 individuals were randomly assigned to a single subcutaneous dose of eplontersen (120 mg) or placebo and 36 individuals were randomly assigned to four doses (every 4 weeks) of either 45 mg, 60 mg or 90 mg eplontersen or placebo[30] (TABLE 1). A single dose of 120 mg eplontersen led to a maximum reduction in serum TTR level from baseline of 86% at

day 29 after dosing. In the multiple-dose cohorts, serum TTR levels decreased by 86%, 91% and 94% 2 weeks after the fourth dose in the 45 mg, 60 mg and 90 mg dosage groups, respectively. Circulating RBP4 levels decreased in parallel with serum TTR levels[30].

An ongoing phase II, single-centre, open-label study on eplontersen is enrolling patients with ATTR-CM who completed the 24 months of the NCT03702829 phase II study on inotersen[72,75]. Patients will be offered to switch to monthly subcutaneous injections of 45 mg eplontersen. Baseline evaluation will include echocardiography, laboratory assessment, 6MWD test, cardiopulmonary exercise testing and, where feasible, CMR. The primary end points are the change from baseline to 48 months in echocardiography-derived longitudinal LV strain, NT-proBNP, high-sensitivity troponin T, 6MWD, maximal oxygen consumption at cardiopulmonary exercise testing, and CMR-derived extracellular volume and LV mass. Study completion is expected in 2025 (REF.[75]).

Phase III clinical trial. Eplontersen is being tested in a phase III trial[76] in patients with stage 1 or stage 2 ATTRv-PN randomly assigned 6:1 to eplontersen 45 mg every 4 weeks or inotersen 300 mg weekly; the placebo group of the NEURO-TTR trial[73] will serve as an external control group. The primary end points are the change from baseline to week 66 in mNIS + 7 and Norfolk QOL-DN. Study completion is expected in 2024 (REF.[76]).

Eplontersen is also being tested in patients with ATTR-CM in an ongoing phase III, multicentre, double-blind, randomized, placebo-controlled study[77]. The study aims to enrol 750 patients by 2024. The primary end point will be a composite of cardiovascular mortality and recurrent cardiovascular clinical events at week 120.

Safety. No serious adverse events were reported in the phase I study[30]. The most common adverse events in patients receiving eplontersen included headache and transient increases in alanine aminotransferase and creatine phosphokinase levels in plasma[30].

CRISPR–Cas9 editing

The CRISPR–Cas9 system is a breakthrough technology that enables targeted in vivo genome editing[78]. A nuclease (Cas9) is complexed with a single-guide RNA, which can be delivered into a specific cell through an appropriate vehicle. Inside the cell, CRISPR–Cas9 induces a double-strand break at a specific genetic location, leading to the absence of production of the target protein (FIG. 1). This technology is being investigated as a possible treatment for multiple human diseases, especially those with a genetic substrate[78]. ATTR amyloidosis is an ideal target for CRISPR–Cas9-mediated gene editing because siRNA-based and ASO-based treatments have demonstrated that *TTR* knockdown is clinically beneficial in patients with ATTR amyloidosis. In addition, *TTR* knockdown through gene silencing does not affect thyroid function and requires only vitamin A supplementation to prevent clinical sequelae related to TTR deficiency. Additionally, plasma TTR is almost entirely produced by hepatocytes, which can be accurately targeted by many delivery systems[79].

In 2018, Finn et al. devised a biodegradable lipid nanoparticle (LNP)-based delivery system to carry a single-guide RNA for in vivo editing via CRISPR–Cas9 (REF.[79]). In the bloodstream, the LNP is covered by apolipoprotein E, which binds to the LDL receptor on the hepatocyte membrane, allowing active endocytosis of the whole delivery system[79]. A single administration of this compound to mice induced a dose-dependent increase in DNA editing in the liver, with decreases of up to 97% in serum TTR levels[79]. This reduction was sustained for 12 months[79]. Similar results were obtained in rats, cynomolgus monkeys and transgenic mice bearing the human Val30Met *TTR* variant, with no significant adverse events[79].

Building on this evidence, Gillmore et al. conducted an open-label, single-dose, phase I study[33] in which two groups of three patients with ATTRv-PN received an intravenous injection of a single-dose regimen (0.1 or 0.3 mg/kg) of NTLA-2001 (an LNP-based CRISPR–Cas9 system for in vivo editing of the *TTR* gene). Patients who previously received RNA-silencing therapy for ATTR amyloidosis were excluded, whereas patients who had previously received TTR stabilizers were eligible after a washout period. Patients were pre-treated with glucocorticoids and antihistamines to avoid inflammatory reactions. At 28 days after the injection, NTLA-2001 treatment was associated with a mean reduction from baseline in circulating TTR levels of 52% and 87% in the low-dose and high-dose regimens, respectively, without any serious adverse events[33]. Therefore, CRISPR–Cas9-mediated gene editing led to a near-complete knockdown of *TTR* expression. This knockdown is expected to be permanent after a single injection of NTLA-2001, and thus has a clear advantage over current siRNA-based and ASO-based therapies, which require serial infusions. Dose escalation is ongoing in this phase I study, and will be followed by expansion of the study cohort (once the optimal dose has been selected) and a 2-year follow-up study. A randomized, phase II–III trial is planned for 2022.

Future directions

Future research should define whether *TTR* silencing has detrimental effects beyond vitamin A deficiency, which can be easily corrected. Both experimental and clinical studies have shown an important neuroprotective activity of TTR: in the preservation and regulation of memory function and behaviour, in response to ischaemic injury, and in nerve regeneration and promotion of neurite outgrowth[80]. Moreover, TTR has been reported to protect against neurodegeneration in experimental models of Alzheimer disease, probably by inhibiting amyloid-β fibril formation[80,81]. Whether long-term treatments that reduce *TTR* expression specifically in the liver will lead to loss of this neuroprotective function requires further investigation. Additionally, metabolic disturbances with *TTR* silencing might result from loss of the central anorectic action of TTR[82] or loss of RBP4 function, which is regarded as an adipokine and a regulator of insulin sensitivity[83,84]. A better understanding of

TTR–RBP4 biology as well as the long-term surveillance of patients receiving *TTR*-silencing therapies will help clarify the possibility of off-target adverse effects.

Finally, reliable and standardized measures of cardiac response to treatment are needed. Changes in LV ejection fraction or mass are not sensitive measures of a cardiac response to treatment[85] and are associated with substantial intraobserver and interobserver variability[85], which is an important limitation when small variations are expected. In patients with cardiac light-chain amyloidosis, LV global longitudinal strain has been associated with cardiac amyloid load[86], and changes in global longitudinal strain have been proposed as indicators of the response to treatment and as outcome predictors[87]. Global longitudinal strain might be considered a tool for longitudinal assessment of patients with ATTR-CM, as previously proposed[26]. Estimated extracellular volume by CMR quantifies the expansion of extracellular spaces by amyloid fibres and possibly fibrosis[60], and high-sensitivity troponins measure the severity of ongoing cardiomyocyte damage and have a low coefficient of variation (that is, even small changes in circulating levels reflect differences in disease activity)[88]. All these measures might be considered surrogate end points in future trials, especially if an association between their change over time and long-term outcomes is established.

Conclusions

Therapies targeting the amyloidogenic cascade are needed to prolong survival and improve the quality of life of patients with ATTR amyloidosis. At present, the only approved drug for ATTR-CM is tafamidis, which stabilizes the TTR tetramer and inhibits the tissue accumulation of amyloidogenic species. For patients with ATTRv-PN, either with or without cardiac involvement, tafamidis, patisiran and inotersen are available options. Complete stabilization of TTR tetramers might be just as effective as complete removal of amyloid fibrils, and we do not yet know which strategy is safer or more effective. Moreover, TTR stabilization agents, such as tafamidis or acoramidis, cannot achieve total stabilization of TTR tetramers and *TTR* knockdown agents do not remove TTR completely.

siRNA therapy drastically reduces circulating TTR levels and shifts the kinetic equilibrium that regulates the formation of TTR amyloid fibres. Accordingly, preliminary data on tafamidis and patisiran show a stabilization of cardiac disease in patients with ATTR-CM receiving tafamidis[25] and cardiac structural and functional improvements in patients with ATTRv amyloidosis with cardiac involvement who were receiving patisiran[26]. The ongoing APOLLO-B[27] and HELIOS-B[67] trials will shed further light on the effects of siRNA therapy on cardiac involvement, including its safety, which was questioned after the discontinuation of the ENDEAVOUR trial[63]. One advantage of siRNA therapy is the formulation, which allows an administration every 3 weeks (for patisiran) or every 3 months (for vutrisiran), compared with the once-daily regimen of tafamidis therapy and the twice-daily regimen of acoramidis. On the basis of their mechanisms of action, *TTR*-targeted siRNA therapy and TTR tetramer stabilizers could hypothetically be combined. However, a clinical trial assessing this combination is extremely unlikely, and no information on this combination will be derived from the APOLLO-B trial given that previous tafamidis treatment is an exclusion criterion[27]. Furthermore, combination therapy will not be cost-effective; tafamidis alone greatly exceeds conventional cost-effectiveness thresholds at the current US list price[89]. Therefore, siRNA and tetramer stabilizer therapies are alternative options, and a careful evaluation of results from completed and ongoing clinical trials will be important to clarify the optimal treatment option.

The evidence on ASOs is limited to the NEURO-TTR trial[73] on inotersen in patients with ATTRv-PN. Moreover, inotersen therapy did not lead to a greater reduction in circulating TTR levels than therapy with the siRNA patisiran, is associated with increased risk of thrombocytopenia and glomerulonephritis, and must be administered every week, which is more frequent than for both patisiran and vutrisiran. Therefore, siRNAs currently seem more promising than the ASO inotersen. Notably, the ASO eplontersen has a more convenient administration schedule than inotersen and seems to be safer based on results from a phase I study[30].

The first results from a CRISPR–Cas9 therapy in patients with ATTRv amyloidosis have provided the intriguing perspective of a true cure for ATTR amyloidosis, with a single administration able to eradicate the source of a progressive, disabling and ultimately fatal disorder[80]. The completed phase I trial is thus eagerly awaited.

In conclusion, tafamidis is the only treatment currently approved for ATTR-CM and ATTRv-PN, and the siRNA patisiran and the ASO inotersen have been approved for the treatment of patients with ATTRv-PN. The siRNAs patisiran and vutrisiran and the ASO eplontersen are being tested in phase III clinical trials in patients with ATTR-CM or ATTRv-PN, whereas gene editing techniques are at an earlier phase of development. An open issue is how to best capture the cardiac response to treatment, and the main foreseeable challenge is the unfavourable cost-effectiveness profile of these drugs.

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