Current and Emerging Therapies for Hereditary Transthyretin Amyloidosis: Strides Towards a Brighter Future

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Abstract

The past few years have witnessed an unprecedented acceleration in the clinical development of novel therapeutic options for hereditary transthyretin amyloidosis. Recently approved agents and drugs currently under investigation not only represent a major breakthrough in this field but also provide validation of the therapeutic potential of innovative approaches, like RNA interference and CRISPR-Cas9-mediated gene editing, in rare inherited disorders. In this review, we describe the evolving therapeutic landscape for hereditary transthyretin amyloidosis and discuss how this highly disabling and fatal condition is turning into a treatable disease. We also provide an overview of the molecular mechanisms involved in transthyretin (TTR) amyloid formation and regression, to highlight how a deeper understanding of these processes has contributed to the identification of novel treatment targets. Finally, we focus on major areas of uncertainty and unmet needs that deserve further efforts to improve long-term patients' outcomes and allow for a brighter future.

Keywords Transthyretin · Gene-silencing · Stabilizers · Amyloidosis · Peripheral neuropathy

A Rapidly Progressing, Severe and Disabling Disease

Hereditary transthyretin amyloidosis (ATTRv) is a dominantly inherited neurodegenerative disorder caused by misfolding and systemic extracellular deposition of variant transthyretin as insoluble amyloid fibrils [1]. More than 130 different *TTR* pathogenic mutations have been reported to date [2]. Although rare, the prevalence of ATTRv is most likely underestimated, particularly in areas where a genetic founder effect is not evident [3]. In these regions, limited disease awareness, phenotypic heterogeneity, incomplete penetrance and variable age at onset are still responsible for a significant rate of misdiagnosis [4].

Early involvement of the peripheral nervous system occurs in association with most pathogenic *TTR* mutations, with Val50Met ATTRv being the prototype of the characteristic axonal, length-dependent, symmetrical sensorimotor polyneuropathy initially described by Corino de Andrade in Portugal in 1952 [5]. Erectile dysfunction, exercise intolerance, resting tachycardia, postural hypotension, sweating abnormalities, xerostomia, urinary disturbances and eye dryness reflect concomitant autonomic neuropathy, which is highly prevalent across different genotypes [6, 7]. Moreover, gastrointestinal manifestations progressing from early satiety, nausea and constipation to recurrent vomiting, refractory diarrhoea and dysphagia result in a rapid deterioration of patients' nutritional status [8, 9].

Heart involvement usually parallels neurological symptoms and should raise the diagnostic suspicion when typical signs of infiltrative cardiomyopathy are recognized on echocardiography or cardiac magnetic resonance (CMR) in a patient with an idiopathic axonal polyneuropathy [10]. Other key features suggestive for ATTRv cardiomyopathy include cardiac uptake on a bone tracer scintigraphy and a persistent increase in the biomarkers NT-proBNP and troponins [11]. If untreated, ATTRv cardiomyopathy invariably progresses towards heart failure with initially preserved ejection fraction. It may also manifest with severe rhythm abnormalities early in the disease course, including atrioventricular blocks and atrial fibrillation. Together with autonomic dysfunction, cardiac involvement is a major prognostic driver in hereditary transthyretin amyloidosis [12]. Mutations associated with a predominant

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cardiac phenotype, including Val142Ile, Thr80Ala and Ile88Leu, are known to result in a worse prognosis, with a median survival of less than 5 years from symptoms' onset [13].

The systemic nature of this multifaceted disease is further emphasized by a wide range of additional signs and symptoms, reflecting renal [14], ligament [15], skeletal muscle [16], spleen [17], eye [18] and leptomeningeal involvement [19].

Neurological progression usually occurs over a few years and results in increasing limitations in performing daily activities, mostly because of profound weakness, pain, numbness and instability in walking. It is now well recognized that the progression of ATTRv polyneuropathy is by far more rapid than other peripheral neuropathies. A multicentre, retrospective study in 283 patients from four countries estimated an annual Neuropathy Impairment Score (NIS) change of 14.3 points in ATTRv [20]. A recent meta-analysis has confirmed this finding and has compared it with the progression of Charcot-Marie-Tooth disease (CMT) and diabetic peripheral neuropathy, showing that the annual deterioration rate in ATTRv exceeds that in other peripheral neuropathies by 12.45 points [21]. Consistently, the substantial disability associated with ATTRv disease progression is captured by the Rasch-built Overall Disability Scale (RODS), which measures activities of daily living and ability to function independently. Baseline data from patients enrolled in the APOLLO trial strongly indicate that significant limitations in performing major everyday activities are already present even in patients in stage 1 polyneuropathy [22]. Considering the debilitating impact of autonomic dysfunction in addition to somatic polyneuropathy and cardiomyopathy, overall the burden on quality of life is profound and significantly worse than in other diseases [22, 23].

From the patients' perspective, this condition has a dramatic emotional, psychosocial and professional burden besides its physical impact [24, 25]. Even patients with mild symptoms frequently report feelings of fear, frustration and sadness, resulting in substantial anxiety and depression [26, 27]. Changes in working life, in personal plans and in family dynamics, together with inability to participate in social activities, significantly contribute to the feelings of loss, guilt, loneliness and uncertainty described by the patients [26, 27].

In this review, we discuss how the past decade has witnessed significant improvement in the management of ATTRv, paralleled by novel insights into the molecular mechanisms of TTR fibrillogenesis and by expanding knowledge on the phenotypic variability and long-term natural history of this disease. Rationale and evidence for available and emerging therapies will be addressed also to highlight the key prognostic relevance of early treatment. Whereas this rapid evolution of the therapeutic landscape is substantially contributing to improving outcomes, there are still several unmet needs.

TTR Amyloidogenesis: a Dynamic Process Between Deposition and Clearance

Expanding knowledge on the molecular mechanisms of TTR amyloidogenesis has significantly contributed to the development of therapeutic strategies for ATTRv and also provides a frame for the interpretation of their effects. It is well-established by the seminal studies of the Scripps' and Porto University's groups that native TTR tetramer disassembly is a critical, rate-limiting step for TTR fibrillogenesis [28, 29]. This key event was initially proved in vitro by showing that TTR dimers and monomers misfold and aggregate following tetramer dissociation under low pH conditions. This model was pivotal in developing the first pharmacological approach to the treatment of ATTRv, represented by small molecule drugs such as tafamidis and diflunisal that increase the kinetic stability of the tetramer and prevent disassembly and aggregation [30].

However, more recent studies are aiming at the identification of the determinants of TTR amyloidogenesis under physiological conditions. This approach stems from the observation that natural amyloid fibrils obtained from ATTR patients' tissues are formed by C-terminal fragments spanning the 49–127 region and full-length TTR monomers, suggesting the in vivo contribution of proteolytic cleavage [31]. In the lack of animal models that adequately recapitulate this disease, it has been recently proposed that the dissociation of the tetramer under physiological conditions is primed by the sequential action of biomechanical forces that are sufficient to destabilize the protein, making native TTR susceptible to selective cleavage by a specific protease [32]. Amyloid fibrils generated through this mechano-enzymatic model are morphologically similar to those extracted from patients, both in terms of classic microscopic characteristics and thermodynamic stability. Forthcoming cryo-electron microscopy analysis will provide further information on the level of ultrastructural similarities between the in vitro and ex vivo fibrils. Moreover, this model reproduces some of the mechanisms that contribute to amyloid formation in vivo, including the role of amyloid seeds in accelerating fibril growth [33].

The role of proteolysis in TTR fibrillogenesis has been additionally advocated in other experimental settings [34], and the identification of the putative enzymes responsible for this process in vivo is ongoing [33, 35]. In particular, plasmin has been shown to reproducibly cleave TTR and generate amyloid fibrils in vitro under physiological conditions, and more recently subtilisin has been also investigated [33, 35].

Pathogenic mutations variably affect TTR aggregation propensity by modulating tetramer kinetic and thermodynamic stability. Mutations localized in the flexible loop connecting strands C and D impart local conformational changes that increase the exposure of regions susceptible to cleavage, accelerating the generation of aggregation-prone, partially unfolded species [36–38]. Whether C-terminal fragments or full-length monomers are more relevant for oligomerization and seeding of pathological aggregates remains to be elucidated. However, the concentration of truncated monomeric species in ex vivo cardiac amyloid fibrils was shown to strongly correlate with the ability to seed further amyloid deposition, suggesting that these intermediates play a key role in the fibrillogenesis pathway [39].

Overall, the kinetics and thermodynamics of TTR aggregation primed by proteases is consistent with a highly dynamic process, potentially modulated in vivo by the contribution of several factors (Fig. 1). These include the intrinsic stability of the TTR tetramer, depending on the specific mutation, and the characteristics of the interstitial milieu crossed by the protein. Whereas some extracellular matrix constituents, such as glycosaminoglycans and serum amyloid P (SAP) component, may act as a scaffold for TTR aggregation, other factors, like extracellular chaperones, may promote the degradation of partially folded intermediate species. Clusterin is overexpressed in TTR amyloid-laden tissues obtained from patients and from transgenic mice carrying human Val50Met in a HSF-1 null background [40, 41]. Moreover, clusterin was shown to interact in vitro with monomeric and oligomeric TTR and to reduce amyloid fibril formation, overall suggesting a role in directing these species to degradation [42].

Additionally, inhibitors or activators of the putative key protease(s) might further contribute to the initiation and progression of the aggregation pathway. In particular, plasminogen activators and inhibitors in the interstitial space are potential candidates as modulators of TTR fibrillogenesis in target tissues. Finally, immune-mediated mechanisms may intervene to promote amyloid reabsorption. Macrophages and fibroblasts internalize and degrade TTR amyloid fibrils and amorphous aggregates in vitro and in wild-type mice [43]. Moreover, metalloproteases (MMPs) released by fibroblasts have a key role in extracellular matrix remodelling. MMPs are upregulated in ATTRv patients' tissues and degrade TTR fibrillar and pre-fibrillar aggregates in vitro [44].

Further elucidation of the mechanisms that modulate TTR fibrillogenesis in vivo may not only lead to the identification of novel therapeutic targets but could also contribute to disclose genetic and/or non-genetic factors that affect the high phenotypic heterogeneity of this disease. Genetic modifiers have long been hypothesized to affect the significant variability in age of onset and disease penetrance observed among patients with Val50Met mutation. Several variants
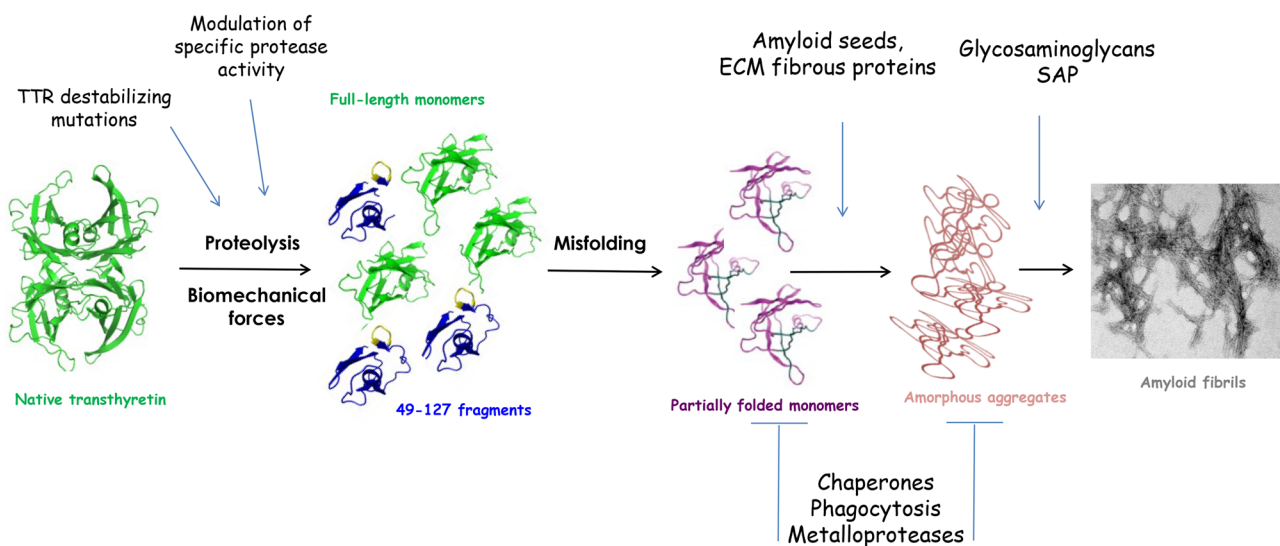

Fig. 1 Main pathogenic events involved in TTR amyloidogenesis. The dissociation of the TTR tetramer is primed by the sequential action of biomechanical forces that are sufficient to destabilize the tetramer and make the protein susceptible to proteolytic cleavage. Full-length monomers and C-terminal fragments are released that are prone to misfolding and aggregation. Several factors may modulate this aggregation pathway, either promoting or inhibiting the generation of a sufficient concentration of partially unfolded species that form the initial amyloid nuclei. Destabilizing mutations, interaction with extracellular matrix components such as fibrous proteins (collagen, elastin), glycosaminoglycans and serum amyloid P component, or pre-existing amyloid deposits are among the factors that promote the process. Extracellular chaperones, matrix metalloproteases and phagocytosis may contribute to the degradation and removal of misfolded species, inhibiting or delaying fibril formation. Abbreviations: SAP, serum amyloid P component; ECM, extracellular matrix

within and outside the TTR locus appeared to correlate with age of onset in the Portuguese population [45, 46]. Recent data further support the role of non-coding variations on TTR gene expression and phenotypic heterogeneity [47, 48]. Moreover, variations in genes affecting functional pathways potentially involved in TTR fibrillogenesis were shown to correlate with age of onset [49, 50]. Finally, epigenetic differences such as DNA methylation changes have also been found in TTR mutation carriers compared to controls, suggesting a potential role in the modulation of TTR amyloidogenesis [51].

Rationale and Evidence for Approved and Emerging Pharmacological Therapies

Treatment of ATTRv should aim at rapidly halting or slowing protein aggregation and amyloid deposition in target tissues to preserve functioning and quality of life, extend survival and possibly reverse organ damage. Therapeutic agents are now available that act on key steps of the TTR amyloidogenic cascade (Fig. 2). These include suppression of circulating native TTR concentration, stabilization of the protein quaternary structure and selective removal of partially folded and aggregated TTR species from plasma and/or tissues.

As expected, the increasing availability of effective pharmacological therapies has been paralleled by a progressive reduction in liver transplantation (OLT) as a treatment option to remove the mutated protein. Moreover, analysis of the long-term outcomes of this approach has highlighted the need for stringent eligibility criteria in order to provide significant survival benefit [52]. Patients with late-onset phenotype, non-Val50Met variants or advanced disease carry a higher risk of rapid disease progression after OLT, mostly related to further deposition of wild-type TTR. Presently, OLT is considered a valuable option in patients with early-onset Val50Met and preserved nutritional status that fail to benefit from pharmacological agents [52].

An early and accurate diagnosis is key for optimal management as loss of neurological function is not generally regained in spite of treatment. In fact, long-term results of open-label extension studies with gene-silencing agents and tafamidis indicate that delayed treatment start is associated with higher disease burden [53–55].

A more widespread and early use of TTR genetic testing to confirm or rule out the diagnosis in symptomatic patients, systematic monitoring of at risk relatives and identification of biomarkers of pre-symptomatic amyloid-related tissue damage are urgent but realistic goals that may significantly contribute to anticipate access to treatment.
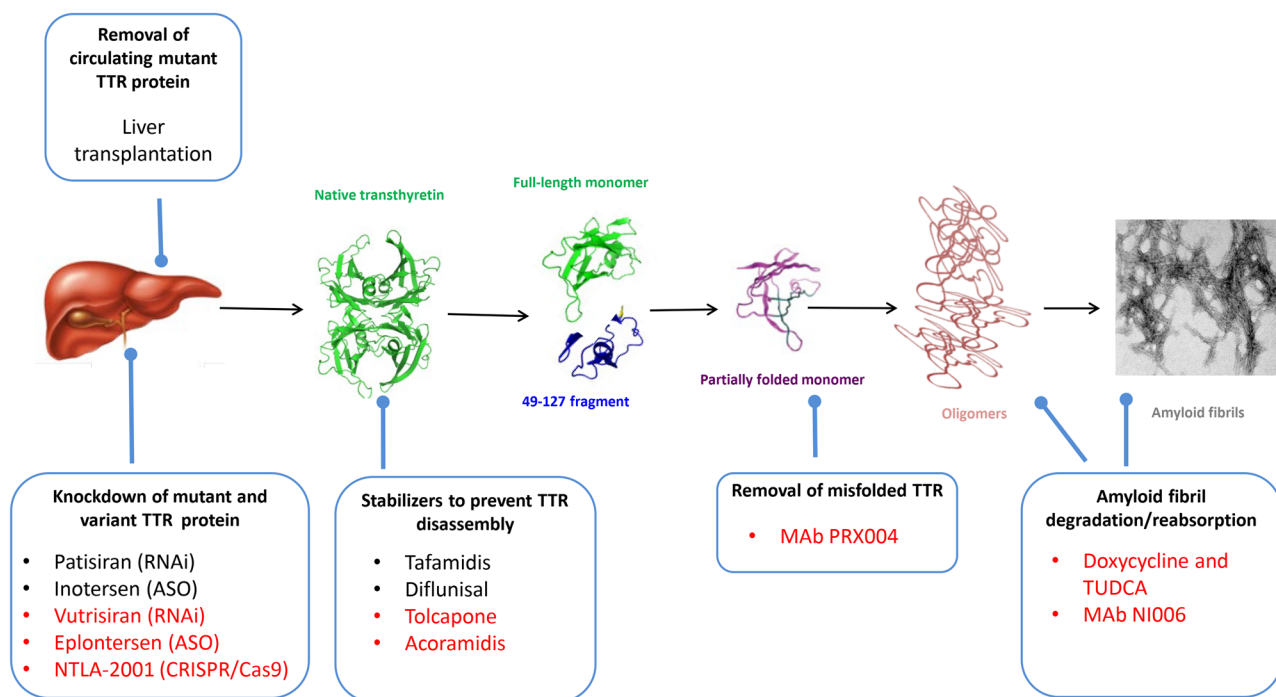

Fig. 2 Current and investigational treatment options for hereditary transthyretin amyloidosis. Approved (black) and investigational (red) therapies for ATTRv are detailed according to their specific target(s).

Abbreviations: mAb, monoclonal antibody, RNAi, RNA interference, ASO, antisense oligonucleotide, TUDCA, tauroursodeoxycholic acid

The efficacy of TTR stabilizers and gene silencing agents for ATTRv has been initially evaluated according to primary and secondary endpoints that measure polyneuropathy progression. Trials assessing cardiac outcome measures have followed and are largely still ongoing. Although a huge amount of information on the natural history of the disease has been gathered thanks to these large but heterogeneous studies, lack of validated criteria for prognostic stratification at enrolment doesn't allow a direct comparison among the different results yet.

Suppressing the Concentration of Circulating Transthyretin

A sustained and substantial reduction in the concentration of the circulating amyloidogenic precursor has proved to be a quite effective approach to the treatment of systemic amyloidosis. In AL amyloidosis, eradication of the plasma cell clone producing the pathogenic immunoglobulin light chain translates into a significant survival benefit and in a high rate of organ responses. The combination of different drugs that synergistically suppress the concentration of the amyloidogenic light chain is now the gold standard for the treatment of this highly aggressive haematological condition [56]. In AA amyloidosis, a persistent reduction of serum amyloid A (SAA) concentration within the lower quartile of the reference level not only strongly correlates with improved renal outcome and survival but may promote regression of amyloid deposits from the liver, the spleen and the kidneys when evaluated by organ biopsy or SAP scintigraphy [57].

In hereditary transthyretin amyloidosis, a significant reduction of both mutant and wild-type plasma TTR can be achieved by different innovative agents that prevent its hepatic secretion either by selective degradation of the protein mRNA [58, 59] or by editing its DNA coding sequence [60].

As TTR plays a major role in the co-transport of vitamin A in plasma through binding to retinol binding protein (RBP), daily vitamin A supplementation is recommended in association with these therapies. No clinical signs of vitamin A insufficiency have emerged until now in patients treated with TTR suppressing agents, but monitoring should be implemented in the absence of a sufficient long-term follow-up.

Antisense Oligonucleotides

Inotersen is the first antisense oligonucleotide (ASO) developed to suppress the hepatic secretion of mutant and wild-type transthyretin. This single-strand 2′-O-methoxyethyl–modified ASO, once internalized by the hepatocytes, enters the nucleus and selectively binds to TTR mRNA, prompting its degradation through cleavage by RNase H. The efficacy and safety of inotersen were evaluated in the pivotal, phase 3 NEURO-TTR trial in which patients were randomized 2:1 to receive either 300 mg inotersen or placebo subcutaneously once a week for 15 months [59]. A 79% median reduction in circulating TTR level was reached in patients treated with inotersen. This rapid and sustained suppression of the amyloidogenic precursor translated into a significant improvement both in neurological progression and in health-related quality of life (HQOL) compared to the placebo group, as assessed by changes from baseline to week 66 in the composite mNIS + 7^{Ionis} score and in Norfolk QOL-DN, respectively [59]. Further analysis of specific aspects of functioning and activities of daily living (ADL) confirmed the beneficial effect of inotersen across the Norfolk domains for ADLs, large fibre/physical functioning and symptoms [61]. Moreover, a higher percentage of inotersen-treated patients showed a clinically meaningful improvement for global measures of physical and mental health according to the SF-36v2 questionnaire compared to placebo [61].

The drug is approved for the treatment of hereditary transthyretin amyloidosis with polyneuropathy (with possible exclusion of FAP stage III patients according to local regulations) in Europe, USA, Japan, Canada and other countries. Differently from patisiran, no corticosteroid-based premedication is required for subcutaneous inotersen injection. However, close monitoring of platelets and renal function are recommended to prevent potentially serious adverse events, i.e. thrombocytopenia and glomerulonephritis that were reported in the pivotal trial in 3 patients each. Recent investigations suggest a possible immune-mediated mechanism underlying thrombocytopenia in some individuals treated with inotersen [62]. Recent immunogenicity assessment in animals and humans showed the occurrence of antidrug antibodies in 30% of patients, but neither an effect on plasma TTR concentration nor possible relationship with onset of adverse events was observed [63].

Patients who completed the NEURO-TTR trial were enrolled in an open-label extension (OLE) study. Long-term data from this study demonstrated the sustained efficacy of inotersen in reducing polyneuropathy progression and preserving quality of life [54]. After a cumulative exposure of 39 months, patients in the inotersen-inotersen group experienced a median 16.98-point change in mNIS + 7^{Ionis}, corresponding to a mean change from OLE baseline of 11.18 points, fully consistent with the benefit observed in this group in the NEURO-TTR trial. Patients switched to inotersen at the OLE baseline showed a sustained improvement in neuropathy progression rate after 24 months, consistent with the response in the inotersen-inotersen arm, according to both mNIS + 7^{Ionis} and Norfolk QOL-DN [54]. However, it is worth noting that the difference in disease burden between the two groups tends to persists over time, highlighting the importance of early diagnosis and treatment.

At data-cut analysis on July 2020, the longest reported exposure to the drug is 6.2 years [64]. Persistent reduction in TTR concentration is maintained, and no grade 4 thrombocytopenia or glomerulonephritis occurred following regular platelet and renal function monitoring. A mild to moderate transient platelet reduction is however commonly observed. After a 3-year period in this extension study, both the inotersen-inotersen group and the placebo-inotersen group continue to demonstrate sustained benefit [64].

Real-word data from long-term experience with inotersen are still limited. In a recent study, Moshe-Lille et al. reported results in nine patients treated with inotersen for neurological progression after liver transplantation [65]. NIS score remained stable or improved over the treatment period (median 12 months) in all patients. However, five patients discontinued the treatment because of reversible side effects.

Eplontersen (ION 288864) is a novel antisense oligonucleotide that is presently under investigation for both ATTRv and wild-type transthyretin amyloidosis (ATTRwt). It belongs to a new platform of chemically-modified ASO that are conjugated to a triantennary N-acetyl galactosamine (GalNAc3) moiety. This mediates asialoglycoprotein receptor-mediated uptake by the hepatocytes maximizing liver targeting, increasing drug potency and allowing for lower and less frequent doses. A recent phase 1 study has confirmed a 30-fold increase in potency of eplontersen compared to inotersen in reducing TTR concentration, with no significant side effects [66]. The safety and efficacy of eplontersen 45 mg, administered subcutaneously once every 4 weeks, is presently investigated in two phase 3 trials. In the NEURO-TTRansform trial (NCT04136184), the change from baseline in mNIS + 7^{Ionis} and Norfolk QOL-DN in patients treated with eplontersen for 15 months will be compared to the historical NEURO-TTR placebo group. Enrolment was recently completed and results are awaited in 2022 [67]. In parallel, eplontersen is under evaluation for either ATTRv or ATTRwt cardiomyopathy in the placebo-controlled phase 3 CARDIO-TTRansform study (NCT04136171).

RNA Interference (RNAi) Therapeutics

Patisiran is the first small interfering RNA (siRNA)-based drug ever approved for the treatment of a human disease. Its clinical development has successfully validated in vivo the therapeutic potential of selective posttranscriptional gene silencing by RNAi. Clinical development of other siRNA is ongoing, and two additional agents, for porphyria [68] and hyperoxaluria type 1, respectively [69], recently received marketing authorization.

RNAi is an endogenous mechanism that regulates gene expression. Synthetic, short double-stranded RNA molecules (siRNA) delivered into cells can trigger the activation of the RNA-inducing silencing complex (RISC) and target the complementary mRNA of interest, promoting its cleavage and degradation and halting the synthesis of its protein product.

Patisiran is a siRNA agent formulated as a lipid nanoparticle (LNP) to protect the short RNA molecules from degradation and to increase targeted delivery to hepatocytes through apolipoprotein E-mediated uptake by LDL receptors. Patisiran is administered every 3 weeks by intravenous infusion at the dose of 0.3 mg/kg, with a maximum 30 mg for patient over 100 kg. Premedication with dexamethasone, acetaminophen and H1- and H2-blockers is associated to prevent injection-related reactions.

The safety and efficacy of patisiran in ATTRv were assessed in the phase 3 APOLLO trial. Significant improvement in neurological progression according to the change from baseline in the primary endpoint mNIS + 7 composite score was observed in patients with polyneuropathy stage 1 and 2 treated with patisiran for 18 months compared to the matched placebo group. Least-squares mean ($\pm$ SE) change from baseline was -6.0 ± 1.7 versus 28.0 ± 2.6 (difference, -34.0 points; $P < 0.001$) [58]. Moreover, 56% of patisiran-treated patients experienced a mNIS + 7 change from baseline less than 0 points compared to the 4% rate observed in the placebo arm. Consistently, 73% of patients had a stable or improved polyneuropathy disability (PND) score in the treatment group, compared to 30% PND stability in the placebo arm, in which no improvement was registered. There were no meaningful differences in efficacy across all subgroups analysed including gender, genotype and FAP stage.

Treatment with patisiran resulted in a median 81% reduction in TTR concentration over 18 months of treatment, with a mean maximum reduction of 87% [70]. TTR reduction in plasma correlates with neurological response [58] although no clear threshold has been established to date that predicts response to therapy.

All secondary endpoints in the APOLLO trial were consistently in favour of patisiran, confirming the significant impact of this treatment on the systemic disease burden. During treatment, quality of life assessed by the total Norfolk QOL-DN score improved relative to baseline in patisiran-treated group, whereas a rapid deterioration was observed in placebo. The difference between the two groups was already evident after 9 months of treatment and across all domains [22]. Further analysis of the efficacy of patisiran in preserving ability to perform daily life activities (ADL) recently showed that a significantly higher proportion of patisiran-treated patients experienced improvement or no change in functioning and social participation compared to placebo according to RODS scale, Karnofsky score and Norfolk ADL domain [71].

Patisiran has received marketing authorization for the treatment of hereditary transthyretin amyloidosis with

polyneuropathy (with possible exclusion of FAP stage III patients according to local regulations) in several countries. Compared to inotersen, no grade 4 serious adverse events were reported in the pivotal trial and no specific blood test monitoring is required. Most common adverse events include mild to moderate injection-related reactions.

The long-term results from the open-label extension study of patisiran confirm the sustained beneficial effect of this treatment. A significant change in the rate of neuropathic progression is observed in patients previously on placebo, with a NIS + 7 change at 12 months fully consistent with that of the patisiran arm in the APOLLO trial [53]. In the most recent analysis reported this year, both patisiran-patisiran and placebo-patisiran patients continue to show stable mNIS + 7 and Norfolk QOL-DN scores after 24 months, and the nutritional status is retained or improved. However, the gap between the two groups measured at the OLE baseline seems to persist, indicating that delayed treatment carries a more pronounced disease burden over time. Interestingly, these results were recently integrated by the observation that the neurofilament light chain (NFL) is reduced in patisiran-treated patients compared to placebo arm at the end of the APOLLO study [72]. Similarly, in the 2-year open-label extension, patients previously on placebo present a significant reduction in NFL, reaching the same concentration as in patients treated with patisiran for an overall 42-month period. These data suggest that this biomarker may effectively reflect response to therapy [73].

The effects of patisiran on cardiac structure and function were investigated in the APOLLO trial in a subpopulation of patients with heart involvement defined according to pre-specified criteria [74]. Treatment with patisiran was associated with reduction in mean left ventricular wall thickness, global and basal longitudinal strain and NT-proBNP concentration compared to placebo [75].

The high number of patients continuing on this study, with few discontinuations reported to date, strengthens the efficacy of patisiran. Long-term exposure remains associated with a very good safety profile. Anti-drug antibodies (ADA) were reported in few cases with no clear clinical impact, overall suggesting that this is not a significant event [70].

Very recently, the results of a 12-month, open label trial with patisiran in patients with ATTRv and polyneuropathy progression after liver transplantation (OLT) were reported [76]. This is the first prospective trial in this delicate patients' population that, together with domino liver-transplanted patients, still represents a significant unmet need. Although the number of patients included in this study is small, either the long disease history (median time from onset 9.4 years, median time from OLT 3.4 years) or the advanced neurological impairment at baseline (PND IIIA/IIIB 56%, mean NIS score 60.3) fully represent the characteristics of this patients' group worldwide.

A median 91% TTR reduction from baseline was observed after 6- and 12-month exposure to patisiran. The safety and tolerability profile is consistent with APOLLO trial. Adverse events were largely mild to moderate, the most common drug-related ones being infusion-related reactions. No discontinuation or death related to study drug was observed. One patient experienced an increase in liver function tests (LFT) and biopsy showed acute liver rejection due to inadequate immunosuppression. Study drug was not skipped. Secondary efficacy endpoints consistently show stabilization of neurological damage over the 12-month treatment period [76].

The novel RNAi therapeutic vutrisiran is presently investigated in two multicentre, randomized, phase 3 studies, namely HELIOS-A for ATTRv-related polyneuropathy and HELIOS-B for cardiomyopathy, including patients with both variant and wild-type ATTR. Vutrisiran belongs to a new class of RNAi agents administered subcutaneously and conjugated to a trivalent GalNAc moiety that specifically binds with high affinity to asialoglycoprotein receptor on the liver. This novel formulation allows for a 3-month interval of administrations. The results of the 9-month primary efficacy analysis from the HELIOS-A study have recently showed that vutrisiran rapidly suppresses TTR concentration similarly to patisiran, with a sustained 83% reduction over the treatment period [77]. In this study, 164 patients were randomized to vutrisiran or patisiran in a 3:1 ratio. Key eligibility criteria and endpoints of HELIOS-A are similar to the APOLLO trial, allowing the placebo arm of this study to serve as the external control for the primary and most secondary endpoints. Vutrisiran met the primary endpoint mNIS + 7 ($p < 0.001$) and achieved statistically significant results ($p < 0.001$) for all planned secondary endpoints (Norfolk QOL-DN and 10-MWT) in the primary efficacy analysis at 9 months. The safety profile is good, with the majority of adverse events being mild in intensity, and no discontinuation or death related to study drug was reported [77].

CRISPR-Cas9 Gene Editing

Besides RNA-targeting agents, sustained lockdown of circulating TTR can be achieved through application of CRISPR-Cas9-mediated gene editing. Preclinical studies in primary human hepatocytes, transgenic human TTR mouse models and cynomolgus monkeys have successfully shown the ability of the therapeutic agent NTLA-2001 to induce selective cleavage of DNA at *TTR* gene sequence, resulting in suppression of TTR serum concentration.

NTLA-2001 consists of two active components, namely a single guide RNA targeting the *TTR* gene and a messenger RNA for Cas9 [60]. These molecules are carried by a lipid nanoparticle that, similarly to other LNP-based delivery systems, is opsonized by apolipoprotein E and specifically

targeted to the hepatocytes, where uptake occurs through LDL receptors. Preliminary results from an ongoing phase 1 clinical trial have recently shown that NTLA-2001 is able to induce the cleavage of the *TTR* gene in a dose-dependent manner, resulting in a 80 to 96% reduction in TTR concentration at the highest dose of 0.3 mg/kg. The safety profile appears favourable, with no reported serious adverse events and only mild side effects observed in three out of six patients. Additional doses and long-term evaluations are expected to confirm the ability of NTLA-2001 to induce a more pronounced and possibly nearly complete TTR knock out.

TTR Stabilizers

Tafamidis

Tafamidis is the first pharmacological therapy ever approved for hereditary TTR amyloidosis. It has initially received marketing authorization for the treatment of ATTRv-related polyneuropathy at the oral dosage of 20 mg daily in several countries, excluding the USA and UK. However, significant heterogeneity in the access to this therapy still exists across countries.

Based on experimental evidence demonstrating that native TTR tetramer dissociation is the critical, rate-limiting step for TTR fibrillogenesis, as discussed before [78], tafamidis was properly designed to target this key pathogenic event [30]. Tafamidis is a small molecule drug that binds with negative cooperativity to the thyroxine binding pockets of TTR, resulting in dose-dependent tetramer kinetic stabilization and inhibition of aggregation in vitro. Following monovalent binding, tafamidis induces conformational changes that result in sufficient tetramer stabilization to inhibit cleavage of the CD loop and monomer dissociation [79]. Tafamidis has high oral bioavailability, good selectivity for TTR in plasma and no effects on thyroid metabolism.

Proof-of-concept evidence of the beneficial effect of TTR stabilization in delaying neurological progression was first obtained in Val50Met patients with Coutinho stage 1 polyneuropathy treated daily with 20 mg tafamidis meglumine in a pivotal, 18-month, placebo-controlled phase 3 trial [80].

In the efficacy-evaluable population, a significant reduction in NIS deterioration and a higher percentage of patients with neuropathy stability was observed in the treatment group compared to placebo. Moreover, prolonged exposure to tafamidis in the two following open-label studies translated in a sustained delay in neurological progression [55, 81]. The highest treatment effect was observed in patients with milder sensorimotor neuropathy at treatment onset, defined by a NIS-LL score below 10 [82]. The long-term tolerability and safety profile of tafamidis is excellent with no significant side effects reported to date [83].

Real-word experience in early-onset Val50Met patients has consistently shown that tafamidis reduces neurological progression, measured by PND score and by a composite neurophysiological score, compared to untreated patients [84]. Moreover, neurological disease stabilization, defined by expert stringent criteria, occurred in 60% of patients in another Portuguese series, being therefore in agreement with the rate reported in the pivotal trial [85]. In the largest series of patients with early-onset Val50Met described to date, tafamidis demonstrated a significant survival advantage compared to a historical untreated group and even in comparison to liver transplantation [86]. Efforts to identify predictors of response to tafamidis have been recently made in order to guide treatment strategy and possibly prevent neurological deterioration in non-responders [85]. Factors that predict response include sex, higher TTR concentration at baseline, higher tafamidis concentration at steady state and lower disease burden at baseline [85]. These predictors have not been validated in any other patients' series to date. However, the prognostic relevance of neurological impairment at baseline appears in agreement with independent observational studies with tafamidis performed in late-onset ATTRv patients presenting a broader range of NIS score. [87–89]. Again, this highlights the key importance of early treatment for better outcome.

Recently, it has been suggested that the concentration of circulating soluble TTR oligomers, in which the protein adopts a non-native conformation, may predict response to tafamidis in early-onset Val50Met ATTRv [90]. In fact, higher pre-treatment plasma concentrations of these non-native TTR structures (NNTTR), measured through a sandwich enzyme-linked immunosorbent assay (ELISA), appear to correlate with poorer response. Further studies are ongoing to validate this as a biological marker in other genotypes and phenotypes.

Both inadequate responses observed in some ATTRv patients and evidence gathered by ex vivo studies in healthy volunteers and wild-type patients [91] have suggested that a higher dose of tafamidis might improve TTR stabilization and translate into better outcomes. This is also consistent with experimental data showing that a higher drug/TTR molar ratio provides a stronger inhibition of TTR cleavage and reduction of amyloid fibril formation in vitro, possibly through a better saturation of the second binding site [92].

Clinical development of the new 80 mg tafamidis dose was started in patients with ATTR cardiomyopathy. In the phase 3, ATTR-ACT clinical trial 441 patients were randomized in a 2:1:2 ratio to receive tafamidis meglumine 80 or 20 mg or placebo for 30 months [93]. Tafamidis significantly reduced all-cause mortality and CV-related hospitalizations compared to placebo, and this effect was statistically significant after 18 months of therapy. Patients treated with tafamidis also presented a reduced decline in functional

capacity according to the 6-min walking test (6MWT) and in health-related quality of life nine months after treatment start [93]. The safety profile of the drug remains unremarkable. Based on these positive results, the drug is approved for the treatment of ATTR cardiomyopathy (hereditary and wild-type) and is presently marketed for this indication as tafamidis free acid 61 mg, that is bioequivalent to 80 mg tafamidis meglumine [94]. In fact, although the ATTR-ACT trial was not powered to compare the efficacy of the two investigated doses, more recent results from the ongoing long-term extension study have provided evidence for the greater survival benefit with tafamidis 80 mg versus 20 mg [95]. Consistently, a greater degree of TTR tetramer stabilization was observed in patients treated with 80 mg [95].

Additional analyses from the ATTR-ACT study indicate that tafamidis improves survival and preserve functioning and quality of life compared to placebo similarly in patients with ATTRwt and ATTRv cardiomyopathy, even if the latter group has a more aggressive disease course and a poorer prognosis when untreated [13]. However, the difference in the mean levels of NT-proBNP was not clinically significant for ATTRv compared to placebo [13]. Consistent with previous observations in ATTRv polyneuropathy, the efficacy of tafamidis appears to be greater in patients with less severe disease at baseline according to NYHA class, further strengthening the importance of early diagnosis [96]. Major advantages of tafamidis are related to its oral bioavailability and an unremarkable safety profile. However, data on the impact of the 61 mg dose on neurological progression in patients with ATTRv mixed phenotype are still lacking.

Diflunisal

The nonsteroidal anti-inflammatory drug diflunisal was investigated as a TTR kinetic stabilizer in a phase 3, investigators-driven, multicentre clinical trial in which the composite score NIS + 7 was proposed for the first time as the primary endpoint to assess neurological progression in ATTRv. The study population is fully representative of the large spectrum of genotypes, phenotypes and disease severity scores observed in these patients, including also polyneuropathy stage 3 [97]. In vitro and ex vivo evidence of significant TTR stabilization achieved by diflunisal at plasma concentrations obtained at standard therapeutic doses provided the rationale for the 250 mg BID dose tested in this study [98].

The Diflunisal Trial met the primary and all secondary endpoints including health-related quality of life and nutritional status measured by the mBMI index. A significant lower change in NIS + 7 score from baseline to month 18 was observed in patients treated with diflunisal compared to placebo. The drug was well-tolerated, with no significant discontinuations or serious adverse events related to it.

Overall, these results successfully repurposed diflunisal for delaying neurological progression in patients with ATTRv polyneuropathy [97].

In spite of its potential gastrointestinal, renal, cardiac and haematological side effects, the use of diflunisal was reported to be safe by additional single-centre experiences [99, 100]. Moreover, both these retrospective series indicated that long-term, real-world therapy with diflunisal was associated with a significant reduction in somatic neurological progression compared to disease natural history and stability in autonomic function parameters, respectively [99, 100]. Presently, diflunisal can be prescribed off-label in several countries. Contraindications include renal failure, concomitant anticoagulant therapy, gastrointestinal bleeding and ischemic cardiomyopathy. Frequent monitoring of renal and cardiac function and blood cell count is recommended.

Moreover, the safety and efficacy of diflunisal for the treatment of ATTRv-related cardiomyopathy have been assessed in a few single-centre, open-label, retrospective studies [101–103]. Although the follow-up is overall relatively short and outcome measures across these series are not comparable, a recent systematic review indicates that treatment with diflunisal in these patients was safe and associated with stability or improvement in cardiac outcomes, including global longitudinal strain (GLS), troponin and overall survival [104].

Acoramidis

Acoramidis (also known as AG10) is a novel small-molecule drug that binds to TTR with higher selectivity than thyroxine or other monovalent TTR stabilizers [105]. Occupancy of thyroxin binding pockets by acoramidis strengthens the interaction at the dimer-dimer interface, stabilizing the tetramer. A phase 2 randomized, placebo-controlled trial has recently evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of acoramidis in patients with ATTR cardiomyopathy and symptomatic heart failure [106]. Patients were treated with two different doses of acoramidis (400 mg or 800 mg twice daily) or placebo for 28 days. The drug was well tolerated with no side effects at both doses. Stabilization of TTR was achieved in patients treated with acoramidis compared to placebo. At the higher dosage, more than 90% TTR stabilization was observed both for mutant and wild-type TTR. The ongoing ATTRibute-CM (NCT 03860935) trial is evaluating the safety and efficacy of acoramidis in patients with symptomatic hereditary or wild-type ATTR cardiomyopathy compared to placebo. Patients were randomized in a 2:1 ratio to receive either acoramidis 800 mg BID or placebo for 30 months. Key primary endpoints are the change from baseline to month 12 in the 6-min walking test (part A of the study) and a hierarchical combination of all-cause mortality and CV-related hospitalization

over the 30-month treatment period. Topline results from Part A of the trial are expected in early 2022. A phase 3, open-label, single-arm study to evaluate the efficacy and safety of acoramidis in patients with ATTR polyneuropathy (ATTRibute-PN Study, NCT04882735) is planned to start soon.

Tolcapone

Repurposing of the catechol-O-methyltransferase inhibitor tolcapone (SOM0226) for the treatment of hereditary ATTR amyloidosis was proposed based on its ability to bind to the T4 binding pockets of TTR with high specificity and without negative cooperativity, strongly inhibiting aggregation of both wild-type and unstable TTR variants [107]. A phase 2a proof-of-concept clinical trial confirmed significant TTR stabilization in patients with ATTR amyloidosis treated with tolcapone with a single 200 mg dose or three 100 mg doses at 4 h intervals [108]. Preclinical data also indicate that the drug is able to stabilize TTR variants associated with leptomeningeal involvement [109]. Combined with its ability to cross the blood–brain barrier, tolcapone warrants clinical development in patients with this particular phenotype, as all other available drugs are known to be unable to reach significant central nervous system (CNS) concentrations.

Treatments Targeting Misfolded and Aggregated TTR

Immunotherapy Agents

The clinical development of therapeutic approaches that target misfolded TTR species, ranging from soluble TTR oligomers to pre-fibrillar and mature amyloid aggregates, represents a new frontier for the treatment of this disease.

The first immunotherapy agent for the clearance of amyloid deposits was developed to target the common amyloid constituent serum amyloid P (SAP) component in tissues, after depletion of its circulating form [110]. However, after the successful proof-of-concept study, a phase 3 trial to evaluate the safety and efficacy of this anti-SAP antibody in patients with cardiac amyloidosis was prematurely discontinued due to side effects.

A novel humanized monoclonal antibody-based therapy is under investigation that specifically targets non-native TTR by binding to an epitope that is exposed only in the misfolded protein conformation. Preclinical studies have shown that the monoclonal antibody (MAb) PRX004 is able to inhibit TTR fibril formation in vitro at low substoichiometric concentrations [111]. Recently, results from an ongoing phase 1 open-label, dose-escalation study with a long-term extension phase (NCT03336580) showed that monthly intravenous infusions of PRX004 are safe and well tolerated across all tested doses. Twenty-one patients with ATTRv cardiomyopathy and/or polyneuropathy were enrolled and completed the dose-escalation phase. Of these, 17 subjects were enrolled in the long-term extension phase. Neurological and cardiac assessment after 9 months of treatment, based on NIS and GLS respectively, demonstrated slowing of neuropathy progression and improved cardiac systolic function in all evaluable patients [112].

Another human-derived antibody targeting pathogenic conformations of TTR, including TTR aggregated forms, oligomers and fibrils, was shown to remove amyloid deposits from patients' cardiac tissues ex vivo and from mice grafted with human fibrils in vivo [113]. Binding to target structures triggers phagocytosis by immune-activated macrophages, promoting amyloid clearance in a dose-dependent manner. Further development of this immunotherapy agent (NI006) is ongoing in a phase 1–2 randomized, placebo-controlled, double-blind, dose escalation trial, followed by an open-label extension phase in subjects with ATTR cardiomyopathy (NCT 04360434).

Doxycycline and TUDCA

Several observations support the anti-amyloidogenic activity of tetracyclines [114, 115]. In particular, doxycycline was shown to disrupt TTR fibrils in vitro and to disaggregate TTR amyloid in transgenic mice [116]. Moreover, combined treatment of doxycycline and tauroursodeoxycholic acid (TUDCA) has shown synergistic effect on removal of TTR deposits in transgenic TTR-FAP mice [117]. The safety profile of doxycycline and TUDCA is well established and favourable. Based on these pre-clinical data, a phase 2 clinical trial of Doxy/TUDCA was conducted in ATTRv (NCT01171859). Treatment was well tolerated and was able to prevent progression of cardiac dysfunction [118]. Doxycycline has been granted Orphan Drug designation by the European Medicine Agency (EMA) for TTR amyloidosis. A randomized placebo-controlled phase 3 trial in wild-type and hereditary cardiac TTR amyloidosis has completed enrolment in 2020, and results are expected in 2022 (NCT03481972).

Treatment Combination and Amyloid Regression

In vivo clearance of amyloid deposits has long been known to occur in patients affected by secondary AA amyloidosis when sustained suppression of the amyloidogenic precursor serum amyloid A is reached. In AA-amyloid target tissues, particularly the liver and the kidneys, this correlates with substantial organ functional improvement. The extracellular matrix components and the cellular effectors

of this process are far from been clarified. Similarly, to which extent this process may occur in vivo in other systemic amyloid diseases and how this may translate into clinical improvement is not defined yet.

Preliminary evidence of possible regression of amyloid in ATTRv is supported by a few clinical observations. Intriguingly, in these studies, patients where mostly treated by the combination of a gene-silencing agent and a TTR stabilizer [119, 120].

In the phase 2, open-label extension study of patisiran in ATTRv, 27 of the 29 patients that completed the previous phase 2 trial [121] continued the treatment for a median of 25 months [119]. As a concomitant TTR stabilizer was allowed in this study, 74% patients were on treatment with either tafamidis or diflunisal at baseline. Sustained reduction of TTR concentration over the treatment period and improvement in mNIS + 7 were reported at 24 months, consistently with the results from the APOLLO OLE study. Interestingly, among exploratory endpoints, quantification of dermal amyloid deposits in skin biopsies was performed by Congo red staining. Significant reduction in the amount of amyloid dermal deposits was observed over a 24-month period. Moreover, sweat gland nerve-fibre density (SGNFD) at both distal thigh and distal leg also improved, suggesting possible autonomic-nerve fibre regeneration. In a recent UK series, 16 patients with ATTRv cardiomyopathy were treated with patisiran over 12 months. Concomitant diflunisal 250 mg twice daily was ongoing in 75% of patients. Treatment outcome was assessed in comparison with a matched historical untreated group. Median serum TTR knockdown in treated patients was 86%, with no difference between patients also receiving diflunisal and those on patisiran alone. Significant reduction in extracellular volume (ECV) mapping was observed on CMR, representing indirect evidence of possible amyloid reabsorption in the myocardium [120]. A significant decrease in NT-proBNP concentration, paralleled by functional improvement according to the 6MWT, was also demonstrated that appears consistent with the benefit reported on cardiac imaging. Additionally, in another single case-report, significant improvement of amyloid deposits in the spleen as well as in the heart was described in a patient treated with patisiran in combination with diflunisal [17].

The clinical benefit of combination approaches certainly requires further investigations. Given actual differences in indication and marketing authorization for gene-silencing agents and tafamidis across several countries and the availability of diflunisal in some areas, it is likely that treatment combination will be increasingly common in daily practice. However, the potential synergic role of these approaches would worth investigation by means of controlled trials.

Towards a Holistic Approach in ATTRv Management

The increasing availability of effective disease-modifying drugs is significantly changing the natural history of ATTRv amyloidosis. Earlier diagnosis and equal access to therapeutic options across different disease stages and phenotypes are key factors for further improving patients' outcomes. Moreover, supportive therapy is not less instrumental for the successful management of this disease. Symptomatic treatment for pain, sexual dysfunction, heart failure, rhythm abnormalities, renal insufficiency, eye problems, low blood pressure and gastrointestinal symptoms provides long-term support to organ dysfunction and contributes to improve quality of life [1]. Nutritional support should be established to counteract inadequate feeding due to gastroparesis, dysphagia, diarrhoea and malabsorption. Physical exercise and physiotherapy are similarly vital for preserving functioning and abilities in daily life activities.

Attention is increasingly paid to establish multidisciplinary teams that are specialized in the care of ATTRv patients and deal with the different medical aspects of this systemic disease [122]. Access to structured multidisciplinary clinics improves patients' adherence to treatment, promotes presymptomatic genetic testing in at risk relatives and motivates asymptomatic carriers to follow-up [123]. Moreover, this approach further increases the skills and the experience of the medical team.

As for the majority of chronic diseases nowadays, multidisciplinary patients' care is mostly devoted to the management of physical aspects, aiming at relieving symptoms and preventing loss of function. However, the profound emotional and psychosocial burden reported by ATTRv patients and their caregivers, even in the early disease stages, calls for a more comprehensive approach to the management of this disease [27]. Together with physical aspects, psychological, social and spiritual needs should deserve more attention. Mental health, emotional well-being, spiritual dimensions and occupational support should be addressed as part of a multidimensional, integrated approach to care that further improves the existing multidisciplinary, but mostly biomedical, models focused on treating the disease symptoms. Active partnership with patients, caregivers and patients advocate groups (PAGs) is fundamental for identifying present unmet needs, gaining novel perspectives and jointly promoting improved long-term standards of care. Finally, the involvement of researchers from humanistic disciplines may additionally provide new knowledge and solutions to develop a holistic approach towards ATTR amyloidosis prevention and care.

Conclusions

Ten years have elapsed since the European Medicine Agency approved tafamidis to delay neurological impairment in patients with stage 1 polyneuropathy [80]. Since then, additional agents have further revolutionized the therapeutic landscape. In parallel, disease awareness has expanded worldwide, with patients being increasingly identified in regions where the disorder was not recognized before. Moreover, a better understanding of the heterogeneous disease phenotypes, contributed also by the results of the Transthyretin Amyloidosis Outcomes Survey (THAOS) registry [124], has improved clinical management globally. Even in the context of the terrible pandemic we are experiencing, the field of ATTR amyloidosis is witnessing an unprecedented acceleration in the number of novel drugs under clinical investigation.

However, this remains a complex, highly disabling and potentially life-threatening disease with several areas of uncertainty and unmet needs. In particular, ocular and central nervous system manifestations are not targeted by existing therapeutic options yet, as all available drugs do not significantly cross the blood–brain barrier. Furthermore, disease-modifying treatments are still initiated upon evidence of amyloid-related tissue damage, although there is increasing evidence that protein deposition and nerve remodelling may start earlier. Lack of biological markers for early diagnosis, disease staging and monitoring response to therapy still limits the management of ATTRv compared to AL amyloidosis. Which is the best timing to start treatment in mutation carriers is one of the key questions that should be addressed by means of properly designed clinical trials. The potential identification of genetic modifiers that predict age of onset and phenotypic presentation might increase our ability to define prognosis and guide a tailored approach in subjects at risk of earlier onset.

As discussed, the therapeutic value of the combination of different therapeutic agents will be increasingly tested in a near future and warrants further investigations in controlled studies. A final consideration concerns the possible contribution of the new gene-silencing and gene-editing therapies to the basic knowledge of our biological systems. Present evidence suggests that TTR plays an essential role only in the brain and likely represents a redundant protein in plasma, particularly in the elderly [125]. Early data obtained in trials using ASO, siRNA or the CRISPR-Cas9 technology support this interpretation to date; however, the long-term clinical monitoring of this lifelong treatment might reveal additional physiological roles of plasma TTR.

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Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

Declarations

Conflict of Interest LO declares speaker honoraria from Pfizer, Alnylam and Akcea. RM declares no conflicts of interest.

References

1. Adams D, Koike H, Slama M, Coelho T. Hereditary transthyretin amyloidosis: a model of medical progress for a fatal disease. Nat Rev Neurol. 2019;15 7:387-404 https://doi.org/10.1038/s41582-019-0210-4.
2. Rowczenio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins PN, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat. 2014;35 9:E2403-12; https://doi.org/10.1002/humu.22619.
3. Schmidt HH, Waddington-Cruz M, Botteman MF, Carter JA, Chopra AS, Hopps M, et al. Estimating the global prevalence of transthyretin familial amyloid polyneuropathy. Muscle Nerve. 2018;57 5:829-37 https://doi.org/10.1002/mus.26034.
4. Cortese A, Vegezzi E, Lozza A, Alfonsi E, Montini A, Moglia A, et al. Diagnostic challenges in hereditary transthyretin amyloidosis with polyneuropathy: avoiding misdiagnosis of a treatable hereditary neuropathy. J Neurol Neurosurg Psychiatry. 2017;88 5:457-8 https://doi.org/10.1136/jnnp-2016-315262.
5. Andrade C. A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain. 1952;75 3:408-27 https://doi.org/10.1093/brain/75.3.408.
6. Tozza S, Severi D, Spina E, Iovino A, Aruta F, Ruggiero L, et al. The neuropathy in hereditary transthyretin amyloidosis: A narrative review. J Peripher Nerv Syst. 2021;26 2:155-9 https://doi.org/10.1111/jns.12451.
7. Gonzalez-Duarte A, Valdes-Ferrer SI, Cantu-Brito C. Characteristics and natural history of autonomic involvement in hereditary ATTR amyloidosis: a systematic review. Clin Auton Res. 2019;29 Suppl 1:1-9 https://doi.org/10.1007/s10286-019-00630-y.
8. Wixner J, Mundayat R, Karayal ON, Anan I, Karling P, Suhr OB, et al. THAOS: gastrointestinal manifestations of transthyretin amyloidosis - common complications of a rare disease. Orphanet J Rare Dis. 2014;9:61 https://doi.org/10.1186/1750-1172-9-61.
9. Obici L, Suhr OB. Diagnosis and treatment of gastrointestinal dysfunction in hereditary TTR amyloidosis. Clin Auton Res. 2019;29 Suppl 1:55-63 https://doi.org/10.1007/s10286-019-00628-6.
10. Adams D, Ando Y, Beirao JM, Coelho T, Gertz MA, Gillmore JD, et al. Expert consensus recommendations to improve diagnosis of ATTR amyloidosis with polyneuropathy. J Neurol. 2021;268 6:2109-22 https://doi.org/10.1007/s00415-019-09688-0.
11. Garcia-Pavia P, Rapezzi C, Adler Y, Arad M, Basso C, Brucato A, et al. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC Working Group on Myocardial and Pericardial Diseases. European heart journal. 2021;42 16:1554-68 https://doi.org/10.1093/eurheartj/ehab072.
12. Gillmore JD, Damy T, Fontana M, Hutchinson M, Lachmann HJ, Martinez-Naharro A, et al. A new staging system for cardiac transthyretin amyloidosis. European heart journal. 2018;39 30:2799-806 https://doi.org/10.1093/eurheartj/ehx589.
13. Rapezzi C, Elliott P, Damy T, Nativi-Nicolau J, Berk JL, Velazquez EJ, et al. Efficacy of Tafamidis in Patients With

Hereditary and Wild-Type Transthyretin Amyloid Cardiomyopathy: Further Analyses From ATTR-ACT. JACC Heart Fail. 2021;9 2:115-23 https://doi.org/10.1016/j.jchf.2020.09.011.

14. Rocha A, Lobato L. Liver transplantation in transthyretin amyloidosis: Characteristics and management related to kidney disease. Transplant Rev (Orlando). 2017;31 2:115-20 https://doi.org/10.1016/j.trre.2016.09.002.

15. Eldhagen P, Berg S, Lund LH, Sorensson P, Suhr OB, Westermark P. Transthyretin amyloid deposits in lumbar spinal stenosis and assessment of signs of systemic amyloidosis. J Intern Med. 2021;289 6:895-905 https://doi.org/10.1111/joim.13222.

16. Carr AS, Pelayo-Negro AL, Jaunmuktane Z, Scalco RS, Hutt D, Evans MR, et al. Transthyretin V122I amyloidosis with clinical and histological evidence of amyloid neuropathy and myopathy. Neuromuscul Disord. 2015;25 6:511-5 https://doi.org/10.1016/j.nmd.2015.02.001.

17. Patel RK, Fontana M, Hawkins PN, Gillmore J. Splenic regression of amyloid on multi-modality imaging in response to treatment with patisiran and diflunisal in hereditary transthyretin amyloidosis. Amyloid. 2021:1–2 https://doi.org/10.1080/13506129.2021.1920389.

18. Beirao JM, Malheiro J, Lemos C, Beirao I, Costa P, Torres P. Ophthalmological manifestations in hereditary transthyretin (ATTR V30M) carriers: a review of 513 cases. Amyloid. 2015;22 2:117-22 https://doi.org/10.3109/13506129.2015.1015678.

19. Maia LF, Magalhaes R, Freitas J, Taipa R, Pires MM, Osorio H, et al. CNS involvement in V30M transthyretin amyloidosis: clinical, neuropathological and biochemical findings. J Neurol Neurosurg Psychiatry. 2015;86 2:159-67 https://doi.org/10.1136/jnnp-2014-308107.

20. Adams D, Coelho T, Obici L, Merlini G, Mincheva Z, Suanprasert N, et al. Rapid progression of familial amyloidotic polyneuropathy: a multinational natural history study. Neurology. 2015;85 8:675-82 https://doi.org/10.1212/WNL.0000000000001870.

21. Lin X, Yarlas A, Vera-Llonch M, Baranwal N, Biber J, Brown D, et al. Rate of neuropathic progression in hereditary transthyretin amyloidosis with polyneuropathy and other peripheral neuropathies: a systematic review and meta-analysis. BMC Neurol. 2021;21 1:70 https://doi.org/10.1186/s12883-021-02094-y.

22. Obici L, Berk JL, Gonzalez-Duarte A, Coelho T, Gillmore J, Schmidt HH, et al. Quality of life outcomes in APOLLO, the phase 3 trial of the RNAi therapeutic patisiran in patients with hereditary transthyretin-mediated amyloidosis. Amyloid. 2020;27 3:153-62 https://doi.org/10.1080/13506129.2020.1730790.

23. Yarlas A, Gertz MA, Dasgupta NR, Obici L, Pollock M, Ackermann EJ, et al. Burden of hereditary transthyretin amyloidosis on quality of life. Muscle Nerve. 2019;60 2:169-75 https://doi.org/10.1002/mus.26515.

24. Magliano L, Obici L, Sforzini C, Mazzeo A, Russo M, Cappelli F, et al. Psychosocial burden and professional and social support in patients with hereditary transthyretin amyloidosis (ATTRv) and their relatives in Italy. Orphanet J Rare Dis. 2021;16 1:163 https://doi.org/10.1186/s13023-021-01812-6.

25. Rintell D, Heath D, Braga Mendendez F, Cross E, Cross T, Knobel V, et al. Patient and family experience with transthyretin amyloid cardiomyopathy (ATTR-CM) and polyneuropathy (ATTR-PN) amyloidosis: results of two focus groups. Orphanet J Rare Dis. 2021;16 1:70 https://doi.org/10.1186/s13023-021-01706-7.

26. Lovley A, Raymond K, Guthrie SD, Pollock M, Sanchorawala V, White MK. Patient-reported burden of hereditary transthyretin amyloidosis on functioning and well-being. J Patient Rep Outcomes. 2021;5 1:3 https://doi.org/10.1186/s41687-020-00273-y.

27. Gonzalez-Moreno J, Gaya-Barroso A, Losada-Lopez I, Rodriguez A, Bosch-Rovira T, Ripoll-Vera T, et al. Val50Met hereditary transthyretin amyloidosis: not just a medical problem, but a psychosocial burden. Orphanet J Rare Dis. 2021;16 1:266 https://doi.org/10.1186/s13023-021-01910-5.

28. Colon W, Kelly JW. Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro. Biochemistry. 1992;31 36:8654-60 https://doi.org/10.1021/bi00151a036.

29. Quintas A, Vaz DC, Cardoso I, Saraiva MJ, Brito RM. Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants. J Biol Chem. 2001;276 29:27207-13 https://doi.org/10.1074/jbc.M101024200.

30. Bulawa CE, Connelly S, Devit M, Wang L, Weigel C, Fleming JA, et al. Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. Proceedings of the National Academy of Sciences of the United States of America. 2012;109 24:9629-34 https://doi.org/10.1073/pnas.1121005109.

31. Bergstrom J, Gustavsson A, Hellman U, Sletten K, Murphy CL, Weiss DT, et al. Amyloid deposits in transthyretin-derived amyloidosis: cleaved transthyretin is associated with distinct amyloid morphology. J Pathol. 2005;206 2:224-32 https://doi.org/10.1002/path.1759.

32. Marcoux J, Mangione PP, Porcari R, Degiacomi MT, Verona G, Taylor GW, et al. A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis. EMBO Mol Med. 2015;7 10:1337-49 https://doi.org/10.15252/emmm.201505357.

33. Mangione PP, Verona G, Corazza A, Marcoux J, Canetti D, Giorgetti S, et al. Plasminogen activation triggers transthyretin amyloidogenesis in vitro. J Biol Chem. 2018;293 37:14192-9 https://doi.org/10.1074/jbc.RA118.003990.

34. Dasari AKR, Arreola J, Michael B, Griffin RG, Kelly JW, Lim KH. Disruption of the CD Loop by Enzymatic Cleavage Promotes the Formation of Toxic Transthyretin Oligomers through a Common Transthyretin Misfolding Pathway. Biochemistry. 2020;59 25:2319-27 https://doi.org/10.1021/acs.biochem.0c00079.

35. Peterle D, Pontarollo G, Spada S, Brun P, Palazzi L, Sokolov AV, et al. A serine protease secreted from Bacillus subtilis cleaves human plasma transthyretin to generate an amyloidogenic fragment. Commun Biol. 2020;3 1:764 https://doi.org/10.1038/s42003-020-01493-0.

36. Mangione PP, Porcari R, Gillmore JD, Pucci P, Monti M, Porcari M, et al. Proteolytic cleavage of Ser52Pro variant transthyretin triggers its amyloid fibrillogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2014;111 4:1539-44 https://doi.org/10.1073/pnas.1317488111.

37. Yee PP, Wei Y, Kim SY, Lu T, Chih SY, Lawson C, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. Nat Commun. 2020;11 1:5424 https://doi.org/10.1038/s41467-020-19193-y.

38. Klimtchuk ES, Prokaeva T, Frame NM, Abdullahi HA, Spencer B, Dasari S, et al. Unusual duplication mutation in a surface loop of human transthyretin leads to an aggressive drug-resistant amyloid disease. Proceedings of the National Academy of Sciences of the United States of America. 2018;115 28:E6428-e36 https://doi.org/10.1073/pnas.1802977115.

39. Saelices L, Chung K, Lee JH, Cohn W, Whitelegge JP, Benson MD, et al. Amyloid seeding of transthyretin by ex vivo cardiac fibrils and its inhibition. Proceedings of the National Academy of Sciences of the United States of America. 2018;115 29:E6741-e50 https://doi.org/10.1073/pnas.1805131115.

40. Greene MJ, Sam F, Soo Hoo PT, Patel RS, Seldin DC, Connors LH. Evidence for a functional role of the molecular chaperone clusterin in amyloidotic cardiomyopathy. Am J Pathol. 2011;178 1:61-8 https://doi.org/10.1016/j.ajpath.2010.11.015.

41. Magalhães J, Saraiva MJ. Clusterin overexpression and its possible protective role in transthyretin deposition in familial

amyloidotic polyneuropathy. J Neuropathol Exp Neurol. 2011;70 12:1097-106 https://doi.org/10.1097/NEN.0b013e31823a44f4.

42. Greene MJ, Klimtchuk ES, Seldin DC, Berk JL, Connors LH. Cooperative stabilization of transthyretin by clusterin and diflunisal. Biochemistry. 2015;54 2:268-78 https://doi.org/10.1021/bi5011249.

43. Misumi Y, Ando Y, Gonçalves NP, Saraiva MJ. Fibroblasts endocytose and degrade transthyretin aggregates in transthyretin-related amyloidosis. Lab Invest. 2013;93 8:911-20 https://doi.org/10.1038/labinvest.2013.83.

44. Sousa MM, do Amaral JB, Guimarães A, Saraiva MJ. Up-regulation of the extracellular matrix remodeling genes, biglycan, neutrophil gelatinase-associated lipocalin, and matrix metalloproteinase-9 in familial amyloid polyneuropathy. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2005;19 1:124–6 https://doi.org/10.1096/fj.04-2022fje.

45. Soares ML, Coelho T, Sousa A, Holmgren G, Saraiva MJ, Kastner DL, et al. Haplotypes and DNA sequence variation within and surrounding the transthyretin gene: genotype-phenotype correlations in familial amyloid polyneuropathy (V30M) in Portugal and Sweden. Eur J Hum Genet. 2004;12 3:225-37 https://doi.org/10.1038/sj.ejhg.5201095.

46. Soares ML, Coelho T, Sousa A, Batalov S, Conceicao I, Sales-Luis ML, et al. Susceptibility and modifier genes in Portuguese transthyretin V30M amyloid polyneuropathy: complexity in a single-gene disease. Hum Mol Genet. 2005;14 4:543-53 https://doi.org/10.1093/hmg/ddi051.

47. Iorio A, De Lillo A, De Angelis F, Di Girolamo M, Luigetti M, Sabatelli M, et al. Non-coding variants contribute to the clinical heterogeneity of TTR amyloidosis. Eur J Hum Genet. 2017;25 9:1055-60 https://doi.org/10.1038/ejhg.2017.95.

48. Alves-Ferreira M, Azevedo A, Coelho T, Santos D, Sequeiros J, Alonso I, et al. Beyond Val30Met transthyretin (TTR): variants associated with age-at-onset in hereditary ATTRv amyloidosis. Amyloid. 2021;28 2:100-6 https://doi.org/10.1080/13506129.2020.1857236.

49. Dias A, Santos D, Coelho T, Alves-Ferreira M, Sequeiros J, Alonso I, et al. C1QA and C1QC modify age-at-onset in familial amyloid polyneuropathy patients. Ann Clin Transl Neurol. 2019;6 4:748-54 https://doi.org/10.1002/acn3.748.

50. Santos D, Coelho T, Alves-Ferreira M, Sequeiros J, Mendonca D, Alonso I, et al. Variants in RBP4 and AR genes modulate age at onset in familial amyloid polyneuropathy (FAP ATTRV30M). Eur J Hum Genet. 2016;24 5:756-60 https://doi.org/10.1038/ejhg.2015.180.

51. De Lillo A, Pathak GA, De Angelis F, Di Girolamo M, Luigetti M, Sabatelli M, et al. Epigenetic profiling of Italian patients identified methylation sites associated with hereditary transthyretin amyloidosis. Clin Epigenetics. 2020;12 1:176 https://doi.org/10.1186/s13148-020-00967-6.

52. Ericzon BG, Wilczek HE, Larsson M, Wijayatunga P, Stangou A, Pena JR, et al. Liver Transplantation for Hereditary Transthyretin Amyloidosis: After 20 Years Still the Best Therapeutic Alternative? Transplantation. 2015;99 9:1847-54 https://doi.org/10.1097/TP.0000000000000574.

53. Adams D, Polydefkis M, Gonzalez-Duarte A, Wixner J, Kristen AV, Schmidt HH, et al. Long-term safety and efficacy of patisiran for hereditary transthyretin-mediated amyloidosis with polyneuropathy: 12-month results of an open-label extension study. Lancet Neurol. 2021;20 1:49-59 https://doi.org/10.1016/S1474-4422(20)30368-9.

54. Brannagan TH, Wang AK, Coelho T, Waddington Cruz M, Polydefkis MJ, Dyck PJ, et al. Early data on long-term efficacy and safety of inotersen in patients with hereditary transthyretin amyloidosis: a 2-year update from the open-label extension

of the NEURO-TTR trial. Eur J Neurol. 2020;27 8:1374-81 https://doi.org/10.1111/ene.14285.

55. Barroso FA, Judge DP, Ebede B, Li H, Stewart M, Amass L, et al. Long-term safety and efficacy of tafamidis for the treatment of hereditary transthyretin amyloid polyneuropathy: results up to 6 years. Amyloid. 2017;24 3:194-204 https://doi.org/10.1080/13506129.2017.1357545.

56. Palladini G, Kastritis E, Maurer MS, Zonder J, Minnema MC, Wechalekar AD, et al. Daratumumab plus CyBorD for patients with newly diagnosed AL amyloidosis: safety run-in results of ANDROMEDA. Blood. 2020;136 1:71-80 https://doi.org/10.1182/blood.2019004460.

57. Lachmann HJ, Goodman HJ, Gilbertson JA, Gallimore JR, Sabin CA, Gillmore JD, et al. Natural history and outcome in systemic AA amyloidosis. N Engl J Med. 2007;356 23:2361-71 https://doi.org/10.1056/NEJMoa070265.

58. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N Engl J Med. 2018;379 1:11-21 https://doi.org/10.1056/NEJMoa1716153.

59. Benson MD, Waddington-Cruz M, Berk JL, Polydefkis M, Dyck PJ, Wang AK, et al. Inotersen Treatment for Patients with Hereditary Transthyretin Amyloidosis. N Engl J Med. 2018;379 1:22-31 https://doi.org/10.1056/NEJMoa1716793.

60. Gillmore JD, Gane E, Taubel J, Kao J, Fontana M, Maitland ML, et al. CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. N Engl J Med. 2021 https://doi.org/10.1056/NEJMoa2107454.

61. Coelho T, Yarlas A, Waddington-Cruz M, White MK, Sikora Kessler A, Lovley A, et al. Inotersen preserves or improves quality of life in hereditary transthyretin amyloidosis. J Neurol. 2020;267 4:1070-9 https://doi.org/10.1007/s00415-019-09671-9.

62. Narayanan P, Curtis BR, Shen L, Schneider E, Tami JA, Paz S, et al. Underlying Immune Disorder May Predispose Some Transthyretin Amyloidosis Subjects to Inotersen-Mediated Thrombocytopenia. Nucleic Acid Ther. 2020;30 2:94-103 https://doi.org/10.1089/nat.2019.0829.

63. Yu RZ, Wang Y, Norris DA, Kim TW, Narayanan P, Geary RS, et al. Immunogenicity Assessment of Inotersen, a 2'-O-(2-Methoxyethyl) Antisense Oligonucleotide in Animals and Humans: Effect on Pharmacokinetics, Pharmacodynamics, and Safety. Nucleic Acid Ther. 2020;30 5:265-75 https://doi.org/10.1089/nat.2020.0867.

64. Coelho: Efficacy and Safety With >3 Years of Inotersen Treatment for the Polyneuropathy of Hereditary Transthyretin Amyloidosis In: European Acad Neurol. 2021.

65. Moshe-Lilie O, Dimitrova D, Heitner SB, Brannagan TH, 3rd, Zivkovic S, Hanna M, et al. TTR gene silencing therapy in post liver transplant hereditary ATTR amyloidosis patients. Amyloid. 2020;27 4:250-3 https://doi.org/10.1080/13506129.2020.1784134.

66. Viney NJ, Guo S, Tai LJ, Baker BF, Aghajan M, Jung SW, et al. Ligand conjugated antisense oligonucleotide for the treatment of transthyretin amyloidosis: preclinical and phase 1 data. ESC Heart Fail. 2021;8 1:652-61 https://doi.org/10.1002/ehf2.13154.

67. Coelho T, Ando Y, Benson MD, Berk JL, Waddington-Cruz M, Dyck PJ, et al. Design and Rationale of the Global Phase 3 NEURO-TTRansform Study of Antisense Oligonucleotide AKCEA-TTR-LRx (ION-682884-CS3) in Hereditary Transthyretin-Mediated Amyloid Polyneuropathy. Neurol Ther. 2021;10 1:375-89 https://doi.org/10.1007/s40120-021-00235-6.

68. Balwani M, Sardh E, Ventura P, Peiro PA, Rees DC, Stolzel U, et al. Phase 3 Trial of RNAi Therapeutic Givosiran for Acute Intermittent Porphyria. N Engl J Med. 2020;382 24:2289-301 https://doi.org/10.1056/NEJMoa1913147.

69. Garrelfs SF, Frishberg Y, Hulton SA, Koren MJ, O'Riordan WD, Cochat P, et al. Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1. N Engl J Med. 2021;384 13:1216-26 https://doi.org/10.1056/NEJMoa2021712.

70. Zhang X, Goel V, Attarwala H, Sweetser MT, Clausen VA, Robbie GJ. Patisiran Pharmacokinetics, Pharmacodynamics, and Exposure-Response Analyses in the Phase 3 APOLLO Trial in Patients With Hereditary Transthyretin-Mediated (hATTR) Amyloidosis. J Clin Pharmacol. 2020;60 1:37-49 https://doi.org/10.1002/jcph.1480.

71. Amanda Peltier AG-D, John Berk, Ivailo Tournev, Ole Suhr, Senda Ajroud-Driss, Madeline Merkel, Hollis Lin, Cecilia Hale, David Adams: Impact of Patisiran on Activities of Daily Living and Functional Status in hATTR Amyloidosis In: AAN. vol. 96: Neurology; 2021.

72. Ticau S, Sridharan GV, Tsour S, Cantley WL, Chan A, Gilbert JA, et al. Neurofilament Light Chain as a Biomarker of Hereditary Transthyretin-Mediated Amyloidosis. Neurology. 2021;96 3:e412-e22 https://doi.org/10.1212/WNL.0000000000011090.

73. Polydefkis: Neurofilament Light Chain (NfL) as a Potential Biomarker of Treatment Response in Hereditary Transthyretin-Mediated Amyloidosis: Data from the Patisiran Global OLE Study In., vol. 96, Neurology edn; 2021.

74. Solomon SD, Adams D, Kristen A, Grogan M, Gonzalez-Duarte A, Maurer MS, et al. Effects of Patisiran, an RNA Interference Therapeutic, on Cardiac Parameters in Patients With Hereditary Transthyretin-Mediated Amyloidosis. Circulation. 2019;139 4:431-43 https://doi.org/10.1161/CIRCULATIONAHA.118.035831.

75. Minamisawa M, Claggett B, Adams D, Kristen AV, Merlini G, Slama MS, et al. Association of Patisiran, an RNA Interference Therapeutic, With Regional Left Ventricular Myocardial Strain in Hereditary Transthyretin Amyloidosis: The APOLLO Study. JAMA Cardiol. 2019;4 5:466-72 https://doi.org/10.1001/jamacardio.2019.0849.

76. Francisco Muñoz-Beamud TC, Julian D Gillmore, Anna Mazzeo, Jonas Wixner, Violaine Planté-Bordeneuve, Laura Lladó, Seth Arum, Matthew T White, Patrick Y Jay, David Adams: Patisiran in Patients with hATTR Amyloidosis Post-Orthotopic Liver Transplant: 12-Month Results. In: Peripheral Nerve Society (PNS) Annual Meeting.

77. David Adams ILT, Mark S Taylor, Teresa Coelho, Violaine Planté-Bordeneuve, John L Berk, Alejandra González-Duarte, Julian D Gillmore, Soon-Chai Low, Yoshiki Sekijima, Laura Obici, Rick Blakesley, Seth Arum, Rebecca Shilling, John Vest, Michael Polydefkis: HELIOS-A: 9-month Results from the Phase 3 Study of Vutrisiran in Patients with Hereditary Transthyretin-Mediated Amyloidosis with Polyneuropathy. In: AAN. vol. 96: Neurology; 2021.

78. Hammarstrom P, Schneider F, Kelly JW. Trans-suppression of misfolding in an amyloid disease. Science. 2001;293 5539:2459-62 https://doi.org/10.1126/science.1062245.

79. Corazza A, Verona G, Waudby CA, Mangione PP, Bingham R, Uings I, et al. Binding of Monovalent and Bivalent Ligands by Transthyretin Causes Different Short- and Long-Distance Conformational Changes. Journal of medicinal chemistry. 2019;62 17:8274-83 https://doi.org/10.1021/acs.jmedchem.9b01037.

80. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Plante-Bordeneuve V, Lozeron P, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology. 2012;79 8:785–92 https://doi.org/10.1212/WNL.0b013e3182661eb1.

81. Coelho T, Maia LF, da Silva AM, Cruz MW, Planté-Bordeneuve V, Suhr OB, et al. Long-term effects of tafamidis for the treatment of transthyretin familial amyloid polyneuropathy. J Neurol. 2013;260 11:2802-14 https://doi.org/10.1007/s00415-013-7051-7.

82. Waddington Cruz M, Amass L, Keohane D, Schwartz J, Li H, Gundapaneni B. Early intervention with tafamidis provides long-term (5.5-year) delay of neurologic progression in transthyretin hereditary amyloid polyneuropathy. Amyloid. 2016;23 3:178–83 https://doi.org/10.1080/13506129.2016.1207163.

83. Huber P, Flynn A, Sultan MB, Li H, Rill D, Ebede B, et al. A comprehensive safety profile of tafamidis in patients with transthyretin amyloid polyneuropathy. Amyloid. 2019;26 4:203-9 https://doi.org/10.1080/13506129.2019.1643714.

84. Conceicao I, Miranda B, Castro J, de Carvalho M. Hereditary amyloidosis related to transthyretin V30M: disease progression in treated and untreated patients. Eur J Neurol. 2018;25 11:1320-e115 https://doi.org/10.1111/ene.13724.

85. Monteiro C, Mesgazardeh JS, Anselmo J, Fernandes J, Novais M, Rodrigues C, et al. Predictive model of response to tafamidis in hereditary ATTR polyneuropathy. JCI Insight. 2019;4 12 https://doi.org/10.1172/jci.insight.126526.

86. Coelho T, Ines M, Conceicao I, Soares M, de Carvalho M, Costa J. Natural history and survival in stage 1 Val30Met transthyretin familial amyloid polyneuropathy. Neurology. 2018;91 21:e1999-e2009 https://doi.org/10.1212/WNL.0000000000006543.

87. Cortese A, Vita G, Luigetti M, Russo M, Bisogni G, Sabatelli M, et al. Monitoring effectiveness and safety of Tafamidis in transthyretin amyloidosis in Italy: a longitudinal multicenter study in a non-endemic area. J Neurol. 2016;263 5:916-24 https://doi.org/10.1007/s00415-016-8064-9.

88. Lozeron P, Theaudin M, Mincheva Z, Ducot B, Lacroix C, Adams D, et al. Effect on disability and safety of Tafamidis in late onset of Met30 transthyretin familial amyloid polyneuropathy. Eur J Neurol. 2013;20 12:1539-45 https://doi.org/10.1111/ene.12225.

89. Plante-Bordeneuve V, Gorram F, Salhi H, Nordine T, Ayache SS, Le Corvoisier P, et al. Long-term treatment of transthyretin familial amyloid polyneuropathy with tafamidis: a clinical and neurophysiological study. J Neurol. 2017;264 2:268-76 https://doi.org/10.1007/s00415-016-8337-3.

90. Jiang X, Labaudiniere R, Buxbaum JN, Monteiro C, Novais M, Coelho T, et al. A circulating, disease-specific, mechanism-linked biomarker for ATTR polyneuropathy diagnosis and response to therapy prediction. Proceedings of the National Academy of Sciences of the United States of America. 2021;118 9 https://doi.org/10.1073/pnas.2016072118.

91. Cho Y, Baranczak A, Helmke S, Teruya S, Horn EM, Maurer MS, et al. Personalized medicine approach for optimizing the dose of tafamidis to potentially ameliorate wild-type transthyretin amyloidosis (cardiomyopathy). Amyloid. 2015;22 3:175-80 https://doi.org/10.3109/13506129.2015.1063485.

92. Verona G, Mangione PP, Raimondi S, Giorgetti S, Faravelli G, Porcari R, et al. Inhibition of the mechano-enzymatic amyloidogenesis of transthyretin: role of ligand affinity, binding cooperativity and occupancy of the inner channel. Sci Rep. 2017;7 1:182 https://doi.org/10.1038/s41598-017-00338-x.

93. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, et al. Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. N Engl J Med. 2018;379 11:1007-16 https://doi.org/10.1056/NEJMoa1805689.

94. Lockwood PA, Le VH, O'Gorman MT, Patterson TA, Sultan MB, Tankisheva E, et al. The Bioequivalence of Tafamidis 61-mg Free Acid Capsules and Tafamidis Meglumine 4 x 20-mg Capsules in Healthy Volunteers. Clin Pharmacol Drug Dev. 2020;9 7:849-54 https://doi.org/10.1002/cpdd.789.

95. Damy T, Garcia-Pavia P, Hanna M, Judge DP, Merlini G, Gundapaneni B, et al. Efficacy and safety of tafamidis doses in the Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT) and long-term extension study. Eur J Heart Fail. 2021;23 2:277-85 https://doi.org/10.1002/ejhf.2027.

96. Perlini S, Mussinelli R, Salinaro F. New effective treatment options reinforce disease awareness: the case of transthyretin cardiac amyloidosis. Eur J Heart Fail. 2021;23 2:290-2 https://doi.org/10.1002/ejhf.2111.

97. Berk JL, Suhr OB, Obici L, Sekijima Y, Zeldenrust SR, Yamashita T, et al. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. JAMA. 2013;310 24:2658-67 https://doi.org/10.1001/jama.2013.283815.

98. Sekijima Y, Dendle MA, Kelly JW. Orally administered diflunisal stabilizes transthyretin against dissociation required for amyloidogenesis. Amyloid. 2006;13 4:236-49 https://doi.org/10.1080/13506120600960882.

99. Sekijima Y, Tojo K, Morita H, Koyama J, Ikeda S. Safety and efficacy of long-term diflunisal administration in hereditary transthyretin (ATTR) amyloidosis. Amyloid. 2015;22 2:79-83 https://doi.org/10.3109/13506129.2014.997872.

100. Takahashi R, Ono K, Shibata S, Nakamura K, Komatsu J, Ikeda Y, et al. Efficacy of diflunisal on autonomic dysfunction of late-onset familial amyloid polyneuropathy (TTR Val-30Met) in a Japanese endemic area. J Neurol Sci. 2014;345 1-2:231-5 https://doi.org/10.1016/j.jns.2014.07.017.

101. Rosenblum H, Castano A, Alvarez J, Goldsmith J, Helmke S, Maurer MS. TTR (Transthyretin) Stabilizers Are Associated With Improved Survival in Patients With TTR Cardiac Amyloidosis. Circ Heart Fail. 2018;11 4:e004769 https://doi.org/10.1161/CIRCHEARTFAILURE.117.004769.

102. Koyama J, Minamisawa M, Sekijima Y, Ikeda SI, Kozuka A, Ebisawa S, et al. Left ventricular deformation and torsion assessed by speckle-tracking echocardiography in patients with mutated transthyretin-associated cardiac amyloidosis and the effect of diflunisal on myocardial function. Int J Cardiol Heart Vasc. 2015;9:1-10 https://doi.org/10.1016/j.ijcha.2015.07.010.

103. Lohrmann G, Pipilas A, Mussinelli R, Gopal DM, Berk JL, Connors LH, et al. Stabilization of Cardiac Function With Diflunisal in Transthyretin (ATTR) Cardiac Amyloidosis. J Card Fail. 2020;26 9:753-9 https://doi.org/10.1016/j.cardfail.2019.11.024.

104. Ibrahim M, Saint Croix GR, Lacy S, Fattouh M, Barillas-Lara MI, Behrooz L, et al. The use of diflunisal for transthyretin cardiac amyloidosis: a review. Heart Fail Rev. 2021 https://doi.org/10.1007/s10741-021-10143-4.

105. Penchala SC, Connelly S, Wang Y, Park MS, Zhao L, Baranczak A, et al. AG10 inhibits amyloidogenesis and cellular toxicity of the familial amyloid cardiomyopathy-associated V122I transthyretin. Proceedings of the National Academy of Sciences of the United States of America. 2013;110 24:9992-7 https://doi.org/10.1073/pnas.1300761110.

106. Judge DP, Heitner SB, Falk RH, Maurer MS, Shah SJ, Witteles RM, et al. Transthyretin Stabilization by AG10 in Symptomatic Transthyretin Amyloid Cardiomyopathy. Journal of the American College of Cardiology. 2019;74 3:285-95 https://doi.org/10.1016/j.jacc.2019.03.012.

107. Sant'Anna R, Gallego P, Robinson LZ, Pereira-Henriques A, Ferreira N, Pinheiro F, et al. Repositioning tolcapone as a potent inhibitor of transthyretin amyloidogenesis and associated cellular toxicity. Nat Commun. 2016;7:10787 https://doi.org/10.1038/ncomms10787.

108. Gamez J, Salvado M, Reig N, Sune P, Casasnovas C, Rojas-Garcia R, et al. Transthyretin stabilization activity of the catechol-O-methyltransferase inhibitor tolcapone (SOM0226) in hereditary ATTR amyloidosis patients and asymptomatic carriers: proof-of-concept study(). Amyloid. 2019;26 2:74-84 https://doi.org/10.1080/13506129.2019.1597702.

109. Pinheiro F, Varejao N, Esperante S, Santos J, Velazquez-Campoy A, Reverter D, et al. Tolcapone, a potent aggregation inhibitor for the treatment of familial leptomeningeal amyloidosis. FEBS J. 2021;288 1:310-24 https://doi.org/10.1111/febs.15339.

110. Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, et al. Therapeutic Clearance of Amyloid by Antibodies to Serum Amyloid P Component. N Engl J Med. 2015;373 12:1106-14 https://doi.org/10.1056/NEJMoa1504942.

111. Higaki JN, Chakrabartty A, Galant NJ, Hadley KC, Hammerson B, Nijjar T, et al. Novel conformation-specific monoclonal antibodies against amyloidogenic forms of transthyretin. Amyloid. 2016;23 2:86-97 https://doi.org/10.3109/13506129.2016.1148025.

112. Suhr: Neurological and cardiac improvements with PRX004 in TTR amyloidosis patients: results of a phase 1 study. In: AAN. vol. 96: Neurology; 2021.

113. Michalon A, Hagenbuch A, Huy C, Varela E, Combaluzier B, Damy T, et al. A human antibody selective for transthyretin amyloid removes cardiac amyloid through phagocytic immune cells. Nat Commun. 2021;12 1:3142 https://doi.org/10.1038/s41467-021-23274-x.

114. Forloni G, Colombo L, Girola L, Tagliavini F, Salmona M. Anti-amyloidogenic activity of tetracyclines: studies in vitro. FEBS letters. 2001;487 3:404-7 https://doi.org/10.1016/s0014-5793(00)02380-2.

115. Diomede L, Cassata G, Fiordaliso F, Salio M, Ami D, Natalello A, et al. Tetracycline and its analogues protect Caenorhabditis elegans from β amyloid-induced toxicity by targeting oligomers. Neurobiol Dis. 2010;40 2:424-31 https://doi.org/10.1016/j.nbd.2010.07.002.

116. Cardoso I, Saraiva MJ. Doxycycline disrupts transthyretin amyloid: evidence from studies in a FAP transgenic mice model. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2006;20 2:234-9 https://doi.org/10.1096/fj.05-4509com.

117. Cardoso I, Martins D, Ribeiro T, Merlini G, Saraiva MJ. Synergy of combined doxycycline/TUDCA treatment in lowering Transthyretin deposition and associated biomarkers: studies in FAP mouse models. J Transl Med. 2010;8:74 https://doi.org/10.1186/1479-5876-8-74.

118. Obici L, Cortese A, Lozza A, Lucchetti J, Gobbi M, Palladini G, et al. Doxycycline plus tauroursodeoxycholic acid for transthyretin amyloidosis: a phase II study. Amyloid. 2012;19 Suppl 1:34-6 https://doi.org/10.3109/13506129.2012.678508.

119. Coelho T, Adams D, Conceição I, Waddington-Cruz M, Schmidt HH, Buades J, et al. A phase II, open-label, extension study of long-term patisiran treatment in patients with hereditary transthyretin-mediated (hATTR) amyloidosis. Orphanet J Rare Dis. 2020;15 1:179 https://doi.org/10.1186/s13023-020-01399-4.

120. Fontana M, Martinez-Naharro A, Chacko L, Rowczenio D, Gilbertson JA, Whelan CJ, et al. Reduction in CMR Derived Extracellular Volume With Patisiran Indicates Cardiac Amyloid Regression. JACC Cardiovasc Imaging. 2021;14 1:189-99 https://doi.org/10.1016/j.jcmg.2020.07.043.

121. Suhr OB, Coelho T, Buades J, Pouget J, Conceicao I, Berk J, et al. Efficacy and safety of patisiran for familial amyloidotic polyneuropathy: a phase II multi-dose study. Orphanet J Rare Dis. 2015;10:109 https://doi.org/10.1186/s13023-015-0326-6.

122. Adams D, Suhr OB, Hund E, Obici L, Tournev I, Campistol JM, et al. First European consensus for diagnosis, management, and treatment of transthyretin familial amyloid polyneuropathy. Curr Opin Neurol. 2016;29 Suppl 1 Suppl 1:S14–26 https://doi.org/10.1097/wco.0000000000000289.

123. Obici L, Kuks JB, Buades J, Adams D, Suhr OB, Coelho T, et al. Recommendations for presymptomatic genetic testing and management of individuals at risk for hereditary transthyretin amyloidosis. Curr Opin Neurol. 2016;29 Suppl 1 Suppl 1:S27–35 https://doi.org/10.1097/wco.0000000000000290.

124. Coelho T, Maurer MS, Suhr OB. THAOS - The Transthyretin Amyloidosis Outcomes Survey: initial report on clinical

manifestations in patients with hereditary and wild-type transthyretin amyloidosis. Curr Med Res Opin. 2013;29 1:63-76 https://doi.org/10.1185/03007995.2012.754348.

125. Liz MA, Coelho T, Bellotti V, Fernandez-Arias MI, Mallaina P, Obici L. A Narrative Review of the Role of Transthyretin in Health and Disease. Neurol Ther. 2020;9 2:395-402 https://doi.org/10.1007/s40120-020-00217-0.