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REVIEW

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Targeting transthyretin - Mechanism-based treatment approaches and future perspectives in hereditary amyloidosis

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Abstract

The liver-derived, circulating transport protein transthyretin (TTR) is the cause of systemic hereditary (ATTRv) and wild-type (ATTRwt) amyloidosis. TTR stabilization and knockdown are approved therapies to mitigate the otherwise lethal disease course. To date, the variety in phenotypic penetrance is not fully understood. This systematic review summarizes the current literature on TTR pathophysiology with its therapeutic implications. Tetramer dissociation is the rate-limiting step of amyloidogenesis. Besides destabilizing *TTR* mutations, other genetic (*RBP4*, *APCS*, *AR*, *ATX2*, *C1q*, *C3*) and external (extracellular matrix, Schwann cell interaction) factors influence the type of onset and organ tropism. The approved small molecule tafamidis stabilizes the tetramer and significantly decelerates the clinical course. By sequence-specific mRNA knockdown, the approved small interfering RNA (siRNA) patisiran and antisense oligonucleotide (ASO) inotersen both significantly reduce plasma TTR levels and improve neuropathy and quality of life compared to placebo. With enhanced

Abbreviations: AD, Alzheimer's disease; ASGPR, asialoglycoprotein receptor; ASO, antisense oligonucleotides; ATTRv, variant-associated transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; BMI, body mass index; CNS, central nervous system; COMT, catechol-O-methyltransferase; CPHPC, R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid; CSF, cerebrospinal fluid; DNA, desoxyribonucleic acid; EGCG, epigallocatechin gallate; EMA, European Medicines Agency; FDA, Food and Drug Administration; GalNac, N-Acetylgalactosamine; GTEx, Genotype-Tissue Expression project; mBMI, modified body mass index; mNIS+7, modified neuropathy impairment score+7 additional items; MOE, 2′-O-methoxyethyl modification; mRNA, messenger ribonucleic acid; NFL, neurofilament light chain; NIS, neuropathy impairment score; NIS-LL, neuropathy impairment score for lower limbs; NorfolkQoL, Norfolk quality of life; NSAID, non-steroidal anti-inflammatory drugs; PND, polyneuropathy disability score; RISC, RNA-induced silencing complex; SAP, serum amyloid P component; siRNA, small-interfering RNA; SNP, single-nucleotide polymorphism; T4, thyroxine; TTR, transthyretin.

hepatic targeting capabilities, GalNac-conjugated siRNA and ASOs have recently entered phase III clinical trials. Bivalent TTR stabilizers occupy both binding groves in vitro, but have not been tested in trials so far. Tolcapone is another stabilizer with the potential to cross the blood–brain barrier, but its half-life is short and liver failure a potential side effect. Amyloid-directed antibodies and substances like doxycycline aim at reducing the amyloid load, however, none of the yet developed antibodies has successfully passed clinical trials. ATTR-amyloidosis has become a model disease for pathophysiology-based treatment. Further understanding of disease mechanisms will help to overcome the remaining limitations, including application burden, side effects, and blood–brain barrier permeability.

KEYWORDS

amyloid-directed antibodies, ATTRv amyloidosis, familial amyloid polyneuropathy (FAP), transthyretin, TTR knockdown, TTR stabilization

1 | INTRODUCTION

Transthyretin (TTR)-related amyloidosis is a progressive, systemic disease caused by dissociation and deposition of the amyloidogenic protein in peripheral sensorimotor and autonomic nerves, heart, vitreous bodies, gastrointestinal mucosae, kidneys, and the central nervous system (Planté-Bordeneuve & Said, 2011). By September 2020, 133 known destabilizing missense variants in the *TTR*-gene (OMIM *176,300) favor this mechanism in an autosomal dominant mode of inheritance (ATTRv amyloidosis). The clinical spectrum is broad and the age of onset variable, ranging from 20 to 80 years for the same variants (Parman et al., 2016). While some mutation carriers remain asymptomatic throughout life, elderly individuals are susceptible to a sporadic wild-type TTR amyloidosis (ATTRwt), predominantly affecting cardiac tissue (Westermark et al., 1990). Although the clinical prevalence of ATTRwt amyloidosis is not clear yet, wild-type TTR amyoid is found in up to 25% of all autopsy cases older than 85 years and might be the underlying cause for heart failure with preserved ejection fraction in up to 13% (Gonzalez-Lopez et al., 2015; Tanskanen et al., 2008). While ATTRv amyloidosis is still considered a rare disease in non-endemic areas, high allele frequencies have been described for the variants p.Val50Met (0.1%) and p.Val144Ile (up to 3.5%) in individuals of northern Portuguese (Plante-Bordeneuve et al., 2003) or west African descent (Buxbaum & Ruberg, 2017). Aside from TTR itself, there must therefore be several genetic and environmental modifiers influencing its amyloidogenicity and organ tropism.

Currently approved treatment options include liver transplantation, thereby removing the main source of mutant TTR in the periphery, but not targeting the 10% produced in the choroid plexus and retina, tetramer stabilization, and TTR mRNA degradation. As primary outcome parameters of all clinical trials, both the overall survival and quality of life were significantly improved under treatment, which is a great success considering the otherwise chronically progressive and fatal disease course. However, each of these approaches has its limitations, and to date, there is no definite cure. In this review, we summarize the underlying mechanisms of both disease and therapy, emphasizing the respective challenges and chances for future studies.

2 | METHODS

For this work, the database (PubMed/Medline) search included the following terms of enquiry "hereditary transthyretin amyloidosis", "TTR amyloidosis", and "familial amyloid polyneuropathy" in combination with "pathophysiology", "TTR function", "TTR stabilization", "TTR knockdown", "antisense oligonucleotides", and "small-interfering RNA". We included peer-reviewed, PubMed-listed publications written in English and published between 1970 and 2020. Registered clinical trials were assessed at clinicaltrials.gov, ISRCTN registry, and clinicaltrialsregister.eu, and respective pharmaceutical companies were contacted requesting informative material. Additional search was based on the authors' expertise on the topic. The final bibliography was generated based on both relevance and originality. We emphasized articles published in the past ten years, however, frequently cited works from previous decades were included as well.

3 | REVIEW

3.1 | From function to disease: understanding TTR amyloidogenicity

3.1.1 | TTR—an amyloidogenic protein

Comprising four exons and 0.4kb, the *TTR* gene is located on chromosome 18q12.1 and encodes a 55 kDa tetramer with four identical monomers of 127 amino acids, each forming a β-sandwich

with one small α-helix and eight β-strands. Following the identification of its amino acid sequence in 1974 (Kanda et al., 1974), the protein was first associated with amyloidosis by Costa and colleagues (Costa et al., 1978). Based on its slightly more negative charge, transthyretin migrates in front of the albumin band in an agarose gel electrophoresis at neutral pH, which is why it was formerly called pre-albumin. The current name "transthyretin" reflects the primary protein function (Liz et al., 2010) of transporting thyroxin (Woeber & Ingbar, 1968) and retinol-binding protein (Smith et al., 1970) in plasma and cerebrospinal fluid (CSF). In humans, contrary to the original nomenclature, TTR is a minor carrier of T4 in plasma (5%–15% of total), whereas the greatest part is carried by thyroxine-binding globulin. Albumin has a lower affinity, but because of its total concentration in serum, the absolute amount of transported T4 may be even greater than that carried by TTR. Therefore, T4 transport can be well compensated in absence of TTR (Liz et al., 2010), whereas retinol-binding protein levels decrease because of a higher renal loss (Liz et al., 2010). Normal concentrations of plasma TTR vary between 20 and 40 mg/dl. As a secretory protein, TTR is assembled and folded in the endoplasmic reticulum, exported to the Golgi, and subsequently secreted for circulation. The main source of TTR synthesis is the liver, contributing roughly 90% of the overall production. Another 10% originate from the choroid plexus and retinal epithelium (Herbert et al., 1986). Representing 20% of the total CSF proteins, TTR levels range between 1.5 and 2.5 mg/dl (Weisner & Roethig, 1983). Especially under neuronal stress, a so far not quantified amount of central TTR production is attributed to neurons, which has been previously discussed as a protective mechanism in Alzheimer's disease (AD) (Ghadami et al., 2020; Li et al., 2011; Wang et al., 2014). How exactly TTR interacts with Aβ and attenuates its toxicity, has so far not been fully understood (for review see (Li & Buxbaum, 2011)).

Within the frame of systemic, TTR-related amyloidosis, RNA-degrading substances have so far not been able to overcome the blood–brain barrier, which is a limitation for the treatment of CNS manifestations. To deprive the CNS of the neuroprotective effects of TTR might, on the other hand, evoke new problems in the future.

3.1.2 | Dissociation and denaturation – steps of amyloidogenesis

Amyloid is an extracellular mass of fibrillar proteins with the typical β sheet structure that can be identified by congo red birefringence or thioflavine S fluorescence staining. To determine the protein of origin, immunohistochemistry (Schönland et al., 2012), immunoelectron microscopy (Arbustini et al., 2002), or mass spectrometry (Winter et al., 2017) are typically applied in specialized laboratories. The rate-limiting step of TTR amyloidogenesis is tetramer dissociation (Colon & Kelly, 1992), which typically takes place at the AB-CD dimer-dimer interface of the homotetramer (Foss et al., 2005). It has been hypothesized in the past that specific proteolytic cleavage of the protomer residues 48–49 located between the C and D strands (Mangione et al., 2018) favors amyloid formation in vitro. Recent ultrastructural characterizations of patient-derived TTR amyloid fibrils showed, however, a preserved continuity of the unfolded full-length monomers, pointing toward the hypothesis that disassembly precedes cleavage in patients (Schmidt et al., 2019), which might therefore be more relevantly associated with aggregation than dissociation. Pathogenic mutations in the *TTR* gene were shown to favor both cleavage and dissociation, so that for example the p.Leu75Pro variant is associated with a 10 times faster disintegration of tetrameric TTR in vitro (Hammarström et al., 2002). Contrarily, the benign variant p.Thr139Met creates additional hydrogen bonds between serine residues of adjacent monomers and therefore increases the tetramer stability, an effect that prevents dissociation in compound-heterozygosity with an otherwise amyloidogenic variant (Almeida et al., 2000).

Depending on denaturating factors and TTR concentrations, monomer unfolding has its own thermodynamic equilibrium, which can be influenced by certain *TTR* variants as well (Hammarström et al., 2002; Hurshman Babbes et al., 2008). Because monomer denaturation is the critical step preceding fibril formation and amyloid deposition, the disease severity and penetrance might not only depend on the tetramer, but also on the monomer stability influenced by both the respectively underlying mutations and by extrinsic modifiers as well.

3.1.3 | Organ tropism, age of onset, and clinical variability—open questions

While the mechanism of amyloidogenesis is well understood, it remains unclear to date, why TTR amyloid deposits in certain, but not in all tissues, contributes to tissue degeneration, and what determines the broad variability in phenotype severity (expressivity) and age at onset (penetrance). It is of special interest, how some carriers of amyloidogenic variants do not develop any symptoms throughout their lifetime, whereas other individuals display a severe cardiomyopathy with thickened walls and TTR-positive deposits without any genetic mutation in *TTR*.

Fragmented TTR has been identified in patient biopsies by protein composition analyses (Ihse et al., 2013). The fragment types, depending on the site of cleavage, correlate with the type of onset and the respective organ tropism (Suhr et al., 2017).

It is further hypothesized that the extracellular matrix might influence both the time and place of protein ensconcing, a mechanism that has been discussed for amyloidosis in general (Kim et al. 2019). By up-regulating remodeling proteins in the presence of TTR fibrils, the tissue microenvironment apparently contributes to neuroinflammation (Sousa et al., 2005). Disturbed axon Schwann cell crosstalk has previously been discussed as a factor in neurodegeneration, a theory established by Sousa and colleagues, showing neuroprotective factors released from Schwann cells are vanishing with disease progression (Sousa, Du Yan, et al., 2001). Recently, Murakami et al.

showed mutant TTR secreted by Schwann cells had an inhibitory effect on neurite outgrowth of sensory neurons in mice, but wild-type TTR did not (Murakami et al., 2015). The role of direct TTR-toxicity imposed on peripheral neurons by their surrounding Schwann cells is, however, unclear to date. It might come into question if hepatic TTR-knockdown alone does not lead to a long-term disease stabilization.

The activation of receptors for advanced glycation end products, responsive to β sheet formation, is hypothesized to induce neuroinflammation. This process is not only associated with yet deposited, but especially with soluble amyloid fibrils (Sousa et al., 2001). Accordingly, small unmyelinated nerve fibers are more and earlier involved despite being less prone to compression than large, myelinated nerves, which goes along with the established theory that the actual amyloid load is of greater extent in proximal nerve sections (Kollmer et al., 2015), still causing a length-dependent neuropathy. Additionally, amyloid vasculopathy has been discussed as another contributor to neuropathy (Koike et al., 2016).

TTR deposition might further be influenced by genetic or epigenetic factors regulating protein expression levels not only of TTR itself, but of other interacting proteins in circulation or tissue. This is supported by the fact that one mutation, namely the most frequent and well-characterized variant p.Val50Met, causes a high-penetrance/early-onset phenotype in the northern Portuguese population, but a low-penetrance/late-onset subtype in other, non-endemic areas (Coelho et al., 2018; Conceição & De Carvalho, 2007; Dohrn et al., 2013; Inês et al., 2018). A notable clinical variability has, however, been described within the same families and, in rare cases, even between monozygotic twins (Ruzhansky et al., 2014; Saporta et al., 2009).

Exploiting the Genotype-Tissue Expression (GTEx) database, Iorio and colleagues hypothesized that the expression of *TTR* mRNA in tissues like skeletal and heart muscle correlates with the geographical distribution of disease manifestations (Iorio, De Angelis, et al., 2017). The same authors identified intronic regulatory variants in the *TTR*-gene that, as they discussed, might influence the regional expression pattern and therefore likewise the tissue susceptibility for amyloidosis (Iorio, De Lillo, et al., 2017). This hypothesis has so far not been confirmed by any experimental data. In comparison to its overall high expression levels in liver, retina, or choroid plexus, *TTR* mRNA is, however, relatively underrepresented in tissues like heart muscle or peripheral nerves (https://bgee.org/?page=gene&gene_id=ENSG00000118271) as well as in cultured cardiomyocytes derived from induced pluripotent stem cells (Synnergren et al., 2008). *TTR*-transgenic mice with cardiac amyloid deposits had no evident transcription of the *TTR* gene in the heart muscle, underlining the primarily non-cardiac origin of TTR amyloid in this model (Buxbaum et al., 2012).

Soares and colleagues (Soares et al., 2005) screened 100 heterozygous p.Val50Met carriers of Portuguese origin, 92 clinically affected (60 early- and 32 late-onset) and 8 pre-symptomatic, for genetic variants in genes associated with TTR-interacting proteins such as *RBP4* and *APCS*. Identifying several single nucleotide polymorphisms (SNP) out of the Hardy–Weinberg equilibrium compared with healthy controls ($n = 85$), the authors assumed that these genes might influence amyloid formation. While the statistical power was too low to show an association with the age of onset for individual candidate genes alone, the combination of several genetic loci including polymorphisms in *RBP4*, *APCS*, *HSPG2*, *ApoE*, *SAA1*, and *SAA2* correlated significantly with the late or early disease manifestation subtype, respectively (Soares et al., 2005). Since that first study, *RBP4* has been reproduced as a modifier by different works (De Lillo et al., 2019; Santos et al., 2016). In a larger Portuguese cohort, including 318 patients from 106 families, for instance, generalized estimating equations of candidate genes revealed three SNPs in *RBP4* associated with late and one with early onset (Santos et al., 2016). By candidate gene screening in 36 early- and 15 late-onset Greek p.Val50Met patients, Dardiotis and colleagues identified polymorphisms in C1Q, a complement cascade protein, and APOε, an apolipoprotein known in the context of Alzheimer's disease, to be associated with an earlier disease onset (Dardiotis et al., 2009). The former association was later reproduced by Dias and colleagues (Dias et al., 2019), whereas the latter could not be confirmed (Satoh et al., 1996; Soares et al., 2005). As assessed by serum protein analyses, Nylander and colleagues had previously reported certain complement factor subtypes to influence the risk of developing a disease phenotype in p.Val50Met *TTR* mutation carriers (Nylander et al., 1990). CAG expansions in the *ATXN2* gene cause autosomal dominant spinocerebellar ataxia if exceeding 32 repeats. In a Portuguese p.Val50Met cohort, heterozygous allele carriers of borderline repeat lengths of at least 22 CAGs showed a significantly earlier age at amyloidosis onset (Santos et al., 2019). Observing that female patients typically present with initial symptoms at a later age than males, and realizing anticipation predominantly occurs in mother-son pairs, Santos and colleagues additionally analyzed the androgen receptor (AR) gene on the X chromosome (Santos et al., 2016). They found five SNPs to be associated with a later or earlier onset with dependence on the patient's sex, respectively (Santos et al., 2016).

Despite these results, all so far performed modifier studies were limited to p.Val50Met mutation carriers and pre-selected candidate genes. Broader genetic screening studies including hereditary and wild-type cases are required for a better understanding of regulating mechanisms derived from yet undiscovered genes or epigenetic factors.

3.2 | On stabilization and knockdown: approved treatment approaches

3.2.1 | Preventing dissociation—small molecules to stabilize the tetramer

To date, the formation of amyloid fibrils can be prevented therapeutically by two different approaches (Figure 1). The first of which, TTR stabilization, exploits the known effect of thyroxine (T4) that enhances the tetramer stability when bound into the

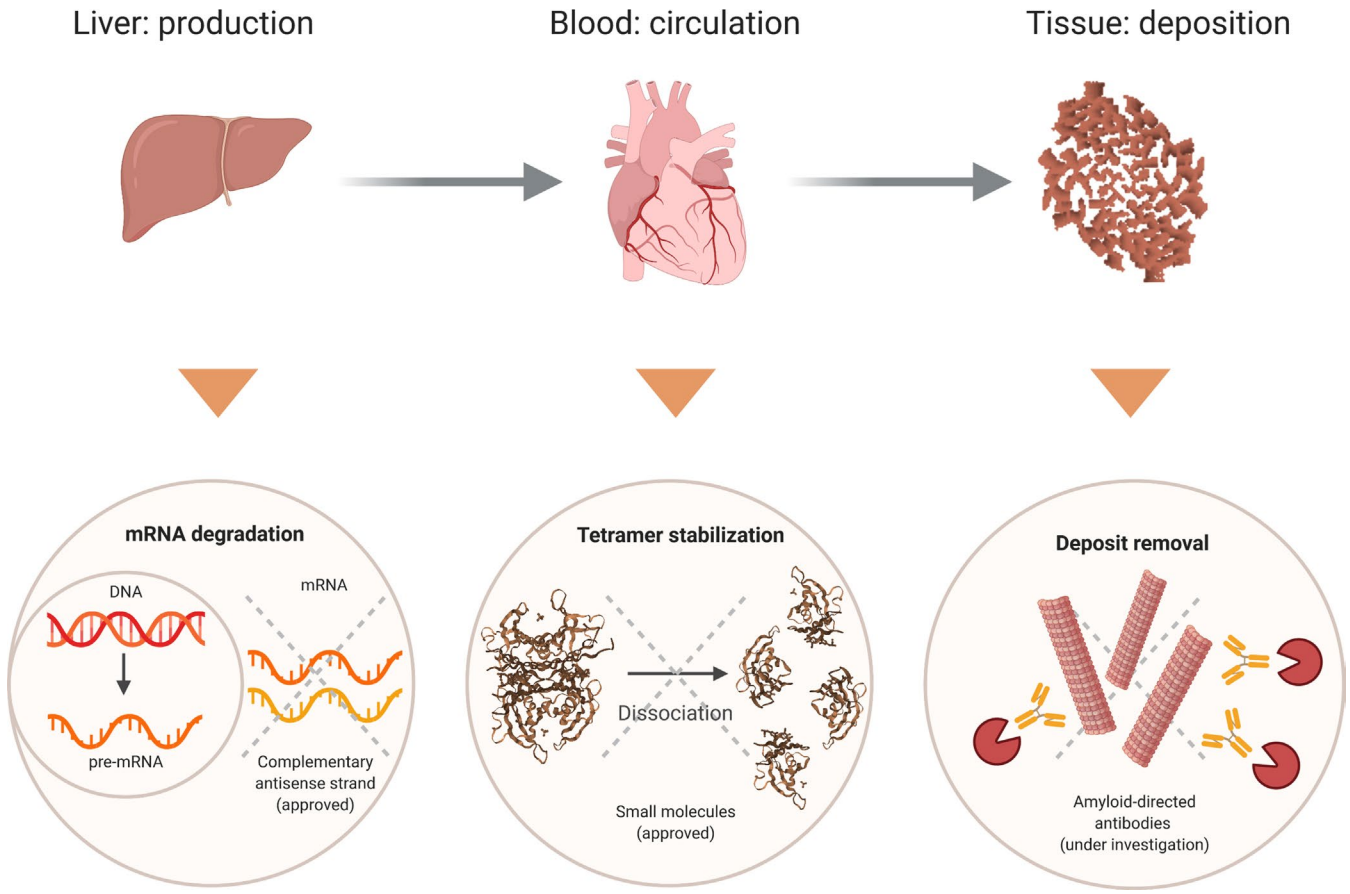

FIGURE 1 Pathophysiology and treatment approaches. Following hepatic production and secretion, the TTR tetramer circulates as a transport protein in blood. Amyloidogenic monomers are drawn towards different tissues including peripheral nerves, heart muscle, and gastrointestinal mucosae, where they form extracellular deposits that stain positive by Congo red. TTR production can be therapeutically targeted by mRNA degrading drugs including the small interference RNA substance patisiran and the antisense oligonucleotide inotersen. Tetramer-stabilizing drugs including tafamidis, diflunisal, and tolcapone prevent TTR dissociation, the first and rate-limiting step of amyloidogenesis. Out of the aforementioned, tafamidis is the only approved substance to date. Amyloid-directed antibodies and substances like doxycycline aim at reducing the deposit load in tissues. They have not been approved for this indication so far.

inter-dimeric grove (Miroy et al., 1996). It has additionally been observed that certain TTR variants do not cause the disease phenotype, but prevent it even in compound-heterozygosity with known, otherwise amyloidogenic mutations (Almeida et al., 2000). This led to the conclusion that the rate-limiting tetramer dissociation can be influenced (Almeida et al., 2005), depending on certain amino acid residues and its interaction. By comparing crystal structures of both TTR and T4, several substances, predominantly non-steroidal anti-inflammatory drugs (NSAID), were identified and tested (Almeida et al., 2005). As the most prominent drug of this group, diflunisal succeeded in a randomized, placebo-controlled clinical trial (NCT00294671) showing significantly reduced neuropathy progression measured by the clinical NIS+7 score (Berk et al., 2013). However, the pre-existing FDA approval of diflunisal for arthritis has never been extended for ATTRv amyloidosis. High drop-out rates in the trial and the risk of cardiac and nephrotoxic side effects (Wixner et al., 2019) have further been points of discussion. Several similarly structured drugs were designed and tested (Miller et al., 2004; Razavi et al., 2003), revealing that 2-(3,5-dichlorophenyl)-1,3-benzoxazole-6-carboxylic acid was most effective in stabilizing the tetramer while being deprived of its cyclooxygenase inhibitory properties. This substance, later called tafamidis, has become the first approved TTR stabilizing drug that entered the European market in a daily oral dosage of 20 mg with an approval for stage 1 ATTRv amyloidosis with polyneuropathy in 2011. The higher dosage of 61 mg received FDA approval for the TTR-associated (ATTRv and ATTRwt) cardiomyopathy in 2019, which was followed by an EMA indication expansion in 2020. The primary outcome parameter of the first, 18 months lasting phase III trial (Fx-005: NCT00409175) was the stop of neuropathy progression that was realized in 60% of the treated compared to 38% of the placebo group (Coelho et al., 2012). Other parameters with a stabilization under treatment were quality of life ($p = .045$ in the efficacy-evaluable group) and the modified body mass index (mBMI, $p < .0001$) (Coelho et al., 2010, 2012). These results were confirmed by an open-label extension trial (Fx-006: NCT00791492), including 71 patients over a time span of 5.5 years (Waddington Cruz et al., 2016). In non-p.

Val50Met patients, a significant TTR stabilization was observed as well, with less evident benefit, however (Merlini et al., 2013). Long-term data on tafamidis (e.g., NCT00925002) revealed about one-third of responders, one-third of partial responders, and one-third of non-responders all defined by progression of sensorimotor and autonomic neuropathy. Positive predictors for a good response aside from being a carrier of the p.Val50Met mutation were female sex and an early disease stage (Monteiro et al., 2019). Focusing on the TTR-related cardiomyopathy, the ATTR-ACT trial (NCT01994889) measured the overall survival and the reduction in cardiovascular events leading to hospitalization and, as secondary outcome parameters, the clinical (6-min walk test) and functional (echocardiography, laboratory parameters) performance under oral tafamidis treatment in a dosage of 20 mg and 80 mg. With an observation time of 30 months, all outcome measures were met for both dosages from the 18 months visit on (Maurer et al., 2018). In all trials, tafamidis was well tolerated with minor side effects including urinary tract infections (for more information, see specialist information).

3.2.2 | Modifying translation—RNA degradation to reduce protein levels

Acknowledging that disease-causing mutations are anchored in the patient's DNA, the concept of "genetic therapy" is particularly attractive. Replacing the main source of mutant TTR protein by liver transplantation became the first mechanism-based treatment of ATTRv amyloidosis that enabled a significant improvement of both span and quality of life (Holmgren et al., 1993; Suhr et al., 1995; Yamashita et al., 2012). Between 1990 and 2010, 1940 patients and 2,127 liver transplants were included in the Familial Amyloidotic Polyneuropathy World Transplant Registry (FAPWTR) (Ericzon et al., 2015), 1628 of whom being carriers of the most frequent Val50Met variant. In 86 cases, the liver was transplanted in combination with other affected organs including heart and/or kidney. In the overall collective, the 20-year survival rate was 55.3% with the greatest benefit in young Val50Met patients. The otherwise healthy liver was donated to patients with end-stage liver diseases in 1,064 of these registered ATTRv cases (Ericzon et al., 2015). This concept, called domino liver transplantation, was first conducted in 1995 (Furtado et al., 1999). In the meantime, several recipients of such an ATTRv liver were diagnosed with an "acquired" form of TTR amyloidosis (Lladó et al., 2010), requiring regular monitoring. Despite longer survival, liver transplantation is not considered a definite cure in liver-transplanted patients with hereditary ATTRv amyloidosis. The slower, but continuous disease progression is attributed to an ongoing deposition of wild-type TTR around the amyloid seeds (Liepnieks et al., 2010). Considering that pre-existing cardiac deposits were the main reason for complications and failure of liver transplant, the combination of both liver and heart transplantation therefore became the therapy of choice in such cases (Barreiros et al., 2010). Unhalted disease progression after liver transplantation was, however, observed in vitreous (Beirão et al., 2015), cerebrovascular (Sekijima, 2015), and leptomeningeal manifestations (Maia et al., 2015). Other challenges including surgery complications, lifelong immunosuppression, and organ allocation problems underline the need for other, less invasive concepts.

Translation modification has become a new therapeutic principle that can be exploited to knock-down the expression of an intended target protein by sequence-specific mRNA degradation. In about 20 years of research, this concept had to overcome pharmacokinetic obstacles such as identifying suitable application pathways and avoiding immediate renal filtration as well as pharmacodynamic challenges including off-target phenomena as well as immune-mediated side effects. With the comparably well-targetable liver in the center of pathogenesis, ATTR amyloidosis has become a model disease for two different classes of expression modification, namely small interfering RNA (siRNA) and antisense oligonucleotides (ASOs). Sharing a similar path of RNA degradation, these two different substance classes harbor some mechanistic differences (Crooke et al., 2018).

ASOs (Crooke et al., 2017) are amphipathic, single-stranded DNA sequences, typically modified at the phosphate and sugar moieties to confer greater exo- and endonuclease resistance. They have a high binding affinity to proteins enabling an easy distribution, which is independent of the route of administration. Various receptors on the hepatocyte cell surface provide their uptake, which is mostly clathrin-, but also caveolin-mediated and partially unspecific. Following endosomal escape, chaperone proteins and GTPases shuttle the substance to the nucleus, where it evolves its highest efficiency in mRNA degradation. The recognition of the target strand is provided by the whole antisense sequence, however, at least two base pairs with unmodified sugar moieties must be accessible to RNase H2, a non-specific endonuclease predominantly found in the nucleus, in order to be recognized as a DNA:RNA hybrid. True to its canonical function, RNase H2 will cleave the phosphodiester bond in the pre-mRNA or mRNA transcript that are bound to DNA, and thereby setting in place a degradation mechanism for the targeted transcript of choice. Sugar moiety modifications can provide greater resistance to endogenous degradation, but these modifications, like the 2′-O-methoxyethyl (MOE) modification routinely used in pre-clinical and clinical models, can create significant steric hindrance, and if used throughout the entire ASO sequence, can block recognition by RNase H2. For this reason, sugar modifications are either reserved for purposes other than transcript knockdown or used in a gapmer fashion, where only the flanking ends of the ASO sequence are modified. The safety of MOE-ASOs, in general, is high, however, thrombopenia has been observed to be caused by different drugs, including inotersen and volanesorsen (Witztum et al., 2019).

Double-stranded siRNA (Setten et al., 2019) molecules range in size between 19 to 21 base pairs with a two-base 3′ overhang. Because of their polyanionic charge and hydrophilic structure, siRNA do not bind to plasma proteins and are therefore prone to rapid renal excretion. To prevent this, lipophilic formulations such as lipid nanoparticles are used enabling an unspecific hepatic uptake by micropinocytosis. They require an intravenous administration and

harbor the potential to cause immunoreactions, so that a pre-medication with corticosteroids and histamine-receptor blockers is necessary (Zhao & Huang, 2014). The mechanism of endosomal escape is not fully understood to date. Once arrived in the cytoplasm, siRNA exploit a natural mechanism of antiviral defense in eukaryotic cells (Coelho et al., 2013), using pre-existing protein complexes for mRNA degradation. Before the antisense sequence can leap into action, however, the sense strand, likewise a "drug-delivery device", has to be removed. Ago2, an argonaut protein complex, recognizes and cleaves RNA and additionally facilitates hybridization with the target mRNA. This induces the formation of the likewise cytoplasmatic located RNA-induced silencing complex (RISC) followed by a sequence-specific degradation of the mRNA of interest.

In the history of translation modification, patisiran, an siRNA molecule, and inotersen, an ASO drug, have competed almost head-to-head to enter the market (Adams et al., 2018; Benson et al., 2018). Reasons that ATTRv became such a pioneer disease are manifold, including the toxic-gain-of-function mechanism that can be addressed by protein reduction, the relative dispensability of TTR in peripheral circulation that makes knockdown a justifiable concept, the hepatic origin of TTR synthesis enabling a systemic application for targeting the organ of interest, and, from an ethical point of view, the particularly cruel and progressive disease character that requires urgent prioritization. As the mechanism is independent of the underlying mutation, it has the potential to treat ATTRv amyloidosis patients with any mutation and to be additionally applied for ATTRwt amyloidosis in the future. As the suppression of circulating TTR protein comes along with reduced vitamin A levels, a daily oral substitution is recommended.

In 2018, **patisiran** reached EMA and FDA approval for the first and second stage of the ATTRv-related polyneuropathy, following an 18-month long, randomized, placebo-controlled phase III trial with 225 participants (APOLLO, NCT01960348) (Adams et al., 2018). It was intravenously administered in a dosage of 0.3 mg per kg body weight every three weeks. The drug led to an 81% (mean) reduction in serum TTR and, as primary endpoint, to a clinical improvement of −6 ± 1 points of the mNIS+7 neuropathy score, whereas placebo-treated patients worsened in a range of 28 ± 2,6 points. An according improvement was measured for the patients' quality of life (Adams et al., 2018; Obici et al., 2020). Cardiac markers including left ventricular hypertrophy, global longitudinal strain, N-terminal pro brain natriuretic peptide, and the 10 meter walk test pointed towards a positive treatment response of the cardiomyopathy as well (Solomon et al., 2019). Typical side effects included peripheral edema and infusion reactions. There were seven deaths in the patisiran (all cardiovascular related) and six in the placebo cohort (three cardiovascular related). In a pooled analysis of cardiac adverse and serious adverse events as well as cardiac hospitalization and death rates, there were no significant differences between the two groups, however (Solomon et al., 2019). Whether the study drug contributed to the development of sudden cardiac deaths or whether these cases were fully attributed to the patients' disease severity has since been the subject of debate (González-Costello et al., 2019). Interim analyses of the not yet published open-label extension trial (NCT02510261), including 211 patients, 49 of which switched from the former placebo arm, confirmed a significant clinical benefit and did not reveal further side effects. The time delay in the former placebo group caused a higher overall disease burden (Polydefkis et al., 2020).

Inotersen was investigated in an international phase III trial including 172 patients in a 2:1 randomization (NEURO-TTR, NCT01737398). By subcutaneous application, patients received a weekly dosage of 284 mg. TTR serum levels were reduced by about 75% (mean) from baseline. As primary endpoints at week 66, the inotersen-treated group showed a significantly lower mNIS+7 score (Benson et al., 2018) and a significantly higher quality of life (Coelho et al., 2019) compared to placebo. These results were independent of the underlying *TTR* variant, the ambulatory status, and the presence of cardiomyopathy (Benson et al., 2018), and led to EMA and FDA approval for the first and second stage of ATTRv-related polyneuropathy in 2018. Subgroup analyses showed a significant reduction in the left ventricular volume and septum diameter (Dasgupta et al., 2020). As mentioned earlier, the most relevant side effect of inotersen was thrombopenia, which might be a class effect as it occurred with volanesorsen, another MOE-ASO, as well (Witztum et al., 2019). Thrombopenia was observed in 60% of all drug-treated patients overall, however, a severe course led to intracranial hemorrhage and death of one study participant. Further common side effects were nausea and vomiting, fever, and glomerulonephritis. Subsequently, the monitoring strategies were improved including obligatory controls of blood cell counts and renal function on a regular basis (for details view specialist information). An open-label extension study (NCT02175004) including 50 previously placebo-treated individuals and 135 patients overall did not reveal any further safety concerns and confirmed a significant benefit especially for the early treated patients (Brannagan 2020 et al.).

3.3 | Overcoming barriers: next steps and novel approaches

3.3.1 | Optimizing administration: GalNac formulations in clinical use

To increase the hepatic delivery (pharmacokinetics) and RNA degradation potency (pharmacodynamics), to therefore enable lower dosages and less frequent application intervals, and additionally to reduce side effects such as infusion (patisiran) or injection site (inotersen) reactions, specific drug modifications are promising. The so far most investigated (Crooke et al., 2019) of such modifications is N-acetylgalactosamine (GalNAc) (Plank et al., 1992), a galactose derivative that binds to the asialoglycoprotein receptor (ASGPR) on hepatocytes (Zimmermann et al., 2017), which leads to a specific, clathrin-mediated uptake. Thus, the same hepatic concentrations can be reached with a drastically reduced dosage. By conjugating the ribose backbone of either ASO or siRNA molecules with GalNAc,

a subcutaneous application is enabled, which is not only easier and more comfortable for the patient, but makes pharmacodynamics slower through a steadier release, decreases renal filtration, and does not require pre-medication in order to reduce infusion reactions (applicable for siRNA only). Several TTR-directed GalNAc-conjugates have been in clinical trials to date (Magrinelli et al., 2020).

Revusiran was the first TTR-directed GalNAc drug to enter clinical trials (Gillmore et al., 2015). Parallel to the APOLLO trial, ENDEAVOUR (NCT02319005) was a placebo-controlled phase III study including 206 2:1 randomized patients with TTR-related cardiomyopathy (Judge et al., 2020). Revusiran, an siRNA molecule like patisiran, was subcutaneously administered in a dosage of 500 mg weekly following a loading interval of five consecutive application days. Primary endpoints were TTR serum levels and the 6-minute walk test. The mean reduction in plasma TTR levels was 80%. Hepatic events occurred in 34.4% of the revusiran-treated compared to 13.6% of the placebo group. Related to heart failure and associated cardiovascular events, 18 patients died in the revusiuran-treated patient cohort. With only 2 death cases in the placebo arm, this imbalance led to a study halt. Subsequent analyses did not reveal any specific causality of the study drug (Judge et al., 2020).

Vutrisiran, a new, "second generation" GalNAc siRNA drug is currently in a patisiran-controlled phase III trial for patients with ATTRv-related polyneuropathy (HELIOS-A, NCT03759379) and in a placebo-controlled phase III trial for patients with hereditary and wild-type cardiac amyoloidosis (HELIOS-B, NCT04153149). With its so called "enhanced stabilization chemistry", vutrisiran is expected to have an up to 10 times increased potency compared with the standard template chemistry of first-generation GalNAc-siRNA. Significant modifications have been made at the sugar-phosphate backbone including the amount and positions of 2'deoxy-2'-fluoro, 2'-O-methyl, and phosphorothioate linkages (Nair et al., 2017). With a dosage of 25 mg every three months, the yearly amount will account for 100 mg (compared to 28 g revusiran per year). A phase 1 trial on 80 healthy subjects, 60 of whom receiving vutrisiran, reported no serious adverse events. TTR plasma levels were reduced by 90%, an effect that lasted for 90 days (2020). The current phase 3 trial, initialized in November 2019, is scheduled until 2025.

In parallel, a GalNAc-modification of inotersen, **AKCEA-TTR-LRx**, seems to be promising to increase the drug's potency and therefore reduce required dosages and side effects. Phase 1 data have not been published to date, however, the safety of several ligand-conjugated antisense drugs appeared to be ensured in several studies including a total of >600 healthy probands (Crooke et al., 2019). In a phase 3 clinical trial initiated in January 2020 (NEURO-TTRansform, NCT04136184), 140 patients with ATTRv-related polyneuropathy are planned to be randomized for subcutaneous injections of either 45 mg AKCEA-TTR-LRx every 4 weeks or 284 mg inotersen once weekly (Khella et al., 2020). For TTR-related hereditary and wild-type cardiac amyloidosis, a parallel placebo-controlled phase III trial (CARDIO-TTRansform, NCT04136171) has begun in March 2020. Both trials are scheduled until 2024.

3.3.2 | Increasing stability beyond borders: new horizons for TTR stabilization

With longer lifespans, new challenges arise, including central nervous system involvement in ATTRv amyloidosis. This experience has already been made in the long-term follow-up of individuals, who underwent liver transplant (Sekijima et al., 2016). About 90% of the circulating TTR in the cerebrospinal fluid is produced in the choroid plexus (Weisner & Roethig, 1983). Hence, targeting the liver by either antisense molecules or transplantation might not be a sufficient long-term concept to prevent CNS manifestations with consequences including stroke-like episodes, dementia, and intracerebral hemorrhage (Nakamura et al., 2005). Blindness can be another disabling manifestation because of retinal TTR production. There is no evidence of TTR degradation in the CNS (Makover et al., 1988). Out of all three approved medications, the small molecule tafamidis is the only one with the potential to cross the blood–brain barrier, however, no more than 1.5% of the plasma-circulating drug actually reach the cerebrospinal fluid, and stabilization kinetics are moderate only (Monteiro et al., 2018).

The orally applicable drug **tolcapone** binds with high affinity to the T4-binding groves. Like tafamidis, it establishes specific contacts with the dimer–dimer interface subsequently reducing the tetramer dissociation of wild-type TTR and several mutants (p. Ala45Thr, p.Val50Met, p.Val50Gly, p. Leu75Pro, p.Tyr134Cys, p.Val142Ile) in vitro. Tolcapone has a similarly stabilizing effect as tafamidis (Sant'Anna et al., 2016), but a greater capacity to cross the blood–brain barrier (Russ et al., 1999). This might be of interest for the treatment of leptomeningeal manifestations of ATTRv amyloidosis in the future (Pinheiro et al., 2020). Because of its short half-life, however, it is difficult to find an efficient dosage for TTR stabilization. With an oral administration of 100 mg (maximum 200 mg) three times daily, the catechol-O-methyltransferase (COMT) inhibitor is already FDA and EMA approved for the treatment of Parkinson's disease. Typical side effects are sleep disturbances, headaches, gastrointestinal symptoms, and elevation of liver enzymes. Because of the increased risk of liver failure, a black box warning has been issued for tolcapone. In cases of liver disease or concomitant drugs sharing the CYP2C9 metabolism (e.g., warfarine), monitoring is required. For ATTRv amyloidosis, tolcapone has passed a phase IIa clinical trial (NCT02191826).

The binding of small ligands by the TTR tetramer significantly reduces its susceptibility to dissociation, cleavage, and aggregation. Full inhibition can only be achieved when both of the two binding groves and the central channel between them are simultaneously occupied (Mangione et al., 2018). When the first pore is filled, this leads to conformational changes in the protein structure, decreasing the accessibility of the second binding grove. Such negative cooperative effects therefore only allow a relatively small percentage of the TTR tetramer to be double stabilized at physiological tafamidis concentrations. Increasing the orally administered dosage accordingly to the ATTR-ACT trial is therefore an attempt that might not fully solve this problem. Another TTR stabilizer, **AG10**, 3-[3-(3,5-dimethyl-1H-pyrazol-4-yl)propoxy]-4-fluorobenzoic acid, is an orally

TABLE 1 Clinical trials mentioned in this review

Trial number/ phase/ publication	Study drug	Duration (months*)	Probands (*n*)	Outcome parameters	Consequences
TTR stabilizers					
NCT00409175/ Fx−005/ III [1, 2]	Tafamidis	18	128	NIS-LL, Norfolk QOL-DN, TQOL, summated 7 score, summated 3 score, mBMI, percentage of participants with stabilized TTR	EMA approval
NCT00791492/ Fx−006/ OLE [3]	Tafamidis	12	86	NIS-LL, TQOL, Norfolk QOL-DN, summated 7 score, summated 3 score, mBMI, troponine I, NT-proBNP, IENF density, percentage of participants with stabilized TTR, SAEs, echocardiographic findings, electrocardiogram findings, holter monitor findings	EMA approval
NCT00925002/OLE	Tafamidis	(ongoing)	93	NIS, TQAL, Karnofsky performance index, PND, AEs, clinical laboratory parameters, electrocardiogram parameters, vital signs, physical examination, concomitant medication	Results expected in 2020
NCT01994889/ ATTR-ACT/ III [4]	Tafamidis	30	441	All-cause mortality, frequency of cardiovascular-related hospitalizations, 6MWT, KCCQ-OS, percentage of participants with stabilized TTR,	FDA approval
NCT00294671/ III [5]	Diflunisal	24	130	NIS+7, Kumamoto neurologic scale, mBMI, SF−36	No approval
NCT02191826/I [6]	Tolcapone	32 hr	17	TTR stabilization, minimal molar ratio, safety	Further investigation
NCT03458130/ II [7]	AG10	28 days	49	AG10 plasma concentration, TTR binding and stabilization	Phase III
NCT03860935/ ATTRIBUTE-CM/ III [8]	AG10	30	510	6MWT, all-cause mortality, cardiovascular-related mortality and hospitalizations, KCCQ, AEs and SAEs, TTR stabilization	Results expected in 2024
TTR silencing drugs					
NCT02319005/ ENDEAVOUR/ III [9]	Revusiran	6.71 months (originally planned for 18 months)	206	6-MWT, TTR levels, cardiovascular mortality, cardiovascular-related hospitalizations, NYHA classification, KCCQ, all-cause mortality	Study halted
NCT01960348/ APOLLO/ III [10, 11, 12]	Patisiran	18	225	mNIS+7, Norfolk QoL-DN, NIS-W, R-ODS, 10-MWT, mBMI, COMPASS 31	EMA and FDA approval
NCT02510261/ OLE	Patisiran	(ongoing)	211	SAEs, NIS, mNIS+7, Norfolk QoL-DN, EQ−5D, COMPASS 31, TTR level, mBMI, R-ODS, NIS-W, 10-MWT, grip strength	Results expected in 2022
NCT03759379/ HELIOS-A/III	Vutrisiran versus. patisiran	(ongoing)	164	mNIS+7, Norfolk QoL-DN, 10-MWT, mBMI, R-ODS, TTR level, all-cause mortality, all-cause hospitalization rate	Results expected in 2024

(Continues)

TABLE 1 (Continued)

Trial number/ phase/ publication	Study drug	Duration (months*)	Probands (*n*)	Outcome parameters	Consequences
NCT04153149/ HELIOS-B/ III	Vutrisiran	(ongoing)	600	All-cause mortality, recurrent cardiovascular events, 6MWT, KCCQ, left ventricular wall thickness, global longitudinal strain, all-cause hospitalizations and urgent visits, NTproBNP	Results expected in 2025
NCT01737398/ NEURO-TTR/ III [13, 14, 15]	Inotersen	15	117	mNIS+7, Norfolk QoL-DN, mBMI, BMI, NIS, GLS, TTR level, RBP4 level	EMA and FDA approval
NCT02175004/OLE	Inotersen	(ongoing)	135	SAEs, blood pressure, heart rate, body weight, routine laboratory panel, QTc, concomitant medications, visual acuity, light detection ability, mNIS+7, NIS, Norfolk QoL-DN, mBMI, PND, TTR level, RBP4 level	Results expected in 2022
NCT04136184/ NEURO-TTRansform/ III	AKCEA-TTR-LRx versus. inotersen	(ongoing)	140	mNIS+7, Norfolk QoL-DN, TTR level, NCS, SF−36, PND, mBMI,	Results expected in 2024
NCT04136171/ CARDIO-TTRansform/ III	AKCEA-TTR-LRx	(ongoing)	750	Cardiovascular mortality, cardiovascular events, 6MWT, KCCQ, all-cause mortality	Results expected in 2024
Amyloid removing drugs					
NCT01677286/ II [16]	Doxycycline	12	25	BNP, troponine I, creatinine clearance, proteinuria	No approval
NCT01171859/ III [17]	Doxycycline+TUDCA	12	40	mBMI, NIS-LL, NT-proBNP, SAEs, Kumamoto scale score, motor and sensory nerve conduction studies, discontinuation rate	No approval
NCT03481972/ OLE	Doxycycline+TUDCA	(ongoing)	102	Overall survival	Results expected in 2021
NCT03336580/I	PRX004	(ongoing)	36	Tolerated dose, adverse events, change in vital parameters, minimal and maximal concentration, $t_{1/2}$, concentration-time curve, anti-drug antibodies	Results expected in 2022

Note: This table provides an exemplary overview of recent and ongoing clinical trials targeting either transthyretin amyloidogenesis, synthesis, or clearance. It summarizes the respective trial durations, numbers of recruited individuals, primary and secondary outcome parameters, and each trial's consequence, for example approval of the study drug, all retrieved from https://clinicaltrials.gov/. If published already, the respective trials are cited in concordance with the manuscript reference list: [1] Coelho et al., 2012; [2] Coelho et al., 2010; [3]Waddington Cruz at al., 2016; [4] Maurer et al., 2018; [5] Berk et al., 2013; [6] Sant'Anna at al., 2016; [7] Fox et al., 2019; [8] Judge et al., 2019; [9] Gillmore et al., 2015; [10] Adams et al., 2018; [11] Solomon et al., 2019; [12] González-Costello et al., 2019; [13] Benson et al., 2018; [14] Coelho et al., 2019; [15] Dasgupta et al., 2020; [16] Obici et al., 2012; [17] Wixner et al., 2017.

10-MWT, 10 meter walk test; 6-MWT, 6-minute walking test; AE, adverse event; BMI, body mass index; COMPASS 31, composite autonomic symptom score; EMA, European medicines agency; EQ-5D, EuroQoL score for quality of life; FDA, food and drug administration; GLS, global longitudinal strain (echocardiogram); IENF density, intraepidermal nerve fiber density; KCCQ, Kansas City Cardiomyopathy Questionnaire; KCCQ-OS, Kansas City Cardiomyopathy Questionnaire Overall Score; mBMI, modified body mass index; mNIS+7, modified NIS score+7 additional items; NCS, neuropathy symptom and change score; NIS, neuropathy impairment score; NIS-LL, neuropathy impairment score for lower limbs; NIS-W, NIS score focusing on weakness; Norfolk QOL-DN, Norfolk quality of life in diabetic neuropathy; NT-proBNP, *N*-terminal prohormone brain natriuretic peptide; NYHA, New York heart association; OLE, open label extension; PND, polyneuropathy disability score; QTc, corrected QT time in electrocardiogram; RBP4, retinol-binding protein; R-ODS, rasch-built overall disability scale; SAE, serious adverse event; SF-36, short form survey; TQAL, total quality of life; TTR, transthyretin; TUDCA, tauroursodeoxycholic acid.

*Indicated if other.

administrable, well-tolerated (Fox et al., 2019) small molecule that provides higher stabilizing propensities by fostering intermonomeric hydrogen bonds comparably to the protective p.Thr139Met variant. Compared to tafamidis and tolcapone, AG10 is slightly more potent to stabilize TTR in patient plasma, to reach the required concentrations, however, patients would have to take 1,600 mg of AG10 versus 80 mg tafamidis per day (Nelson et al., 2020). An ongoing placebo-, but not tafamidis-controlled phase III clinical trial (NCT03860935/ATTRIBUTE-CM), including 510 individuals with cardiac ATTR amyloidosis, is expected to show results in 2024 (Judge et al., 2019). Another approach targeting TTR dissociation are bivalent TTR stabilizers (Corazza et al., 2019; Verona et al., 2017), such as the substance **mds84**, that bind both binding groves and the central protein channel at the same time and therefore exhibit one kinetic binding curve only. By doing so, TTR dissociation and cleavage are both more efficiently prevented compared to monovalent substances including tafamidis and tolcapone in vitro (Corazza et al., 2019; Verona et al., 2017). So far, however, mds84 has not been tested in clinical trials.

Epigallocatechin gallate (EGCG), a polyphenolic component of green tea, binds and stabilizes TTR outside the thyroxine-binding grove. By direct interaction with amino acid residues at the dimer-dimer interface (Miyata et al., 2010), it prevents tetramer dissociation and disrupts preformed amyloid fibrils in vitro (Ferreira et al., 2012). In patients, oral EGCG was shown to be safe (Cappelli et al., 2018) and to reduce left ventricular wall thickness (aus dem Siepen et al., 2015). However, the interpretation of findings was limited by small case numbers or retrospective data acquisition. A prospective, randomized, and placebo-controlled trial on EGCG in patients with light chain amyloidosis (TAME-AL, NCT02015312) did not reveal a significant benefit (data not published).

3.3.3 | Improving amyloid clearance: antibodies to reduce the deposit load

The very first, historic treatment approach was selective plasmapheresis, intending to wash out the amyloidogenic protein (Regnault et al., 1992). This was, however, not successful. Novel therapies aim likewise at removing already deposited amyloid (Müller et al., 2020) (Figure 1). Despite extensive and expensive effort, clearing amyloid has so far been of very limited success.

The tetracycline derivates **doxycycline**, an approved antibiotic drug, has been shown to disrupt amyloid aggregates in mice (Cardoso et al., 2003). Minocycline, a similar drug from the same family as doxycycline, reduces the synthesis of aggregation-prone proteins by targeting cytoplasmatic ribosomes (Solis et al. 2018), a mechanism that could, for doxycycline as well, additionally contribute to the reduction in amyloid load. Biliary acids such as **tauroursodeoxycholic acid** reduce the load of circulating, fibrillar TTR by a not yet fully understood mechanism (Cardoso et al., 2010). In order to prevent and reduce the amyloid load, several combinations of orally administered doxycycline and different biliary acids have been tested in open-label clinical trials (NCT01677286, NCT01171859). Results point towards a clinical benefit for both neurologic and cardiac outcome parameters (Obici et al., 2012; Wixner et al., 2017). Because of high drop-out rates, however, these trials have not led to approval yet. A new phase III trial for the combination of doxycycline and tauroursodeoxycholic acid in patients with cardiac ATTR amyloidosis (NCT03481972) is still ongoing, and results are expected in 2021.

Immediate TTR-directed antibodies (George et al., 2019) were shown to recognize specific epitopes in the fibrillar TTR forms and not to target native TTR in-vitro (Ando & Ueda, 2017). An ongoing phase 1 clinical trial on PRX004 (NCT03336580) has recently finished its recruitment, and results are expected to be published in 2021.

By complement activation and the induction of phagocytosis, antibodies against serum amyloid A component (SAP) aimed at triggering the elimination of systemic amyloid deposits (Bodin et al., 2010; Richards et al., 2015), which seemed to be a promising approach in a previous study including patients with different types of amyloidosis (NCT03336580) (Richards et al., 2018). In a phase 2 clinical trial (NCT03044353) with patients with cardiac ATTR amyloidosis in one and with post-chemotherapy light chain amyloidosis in a second study arm, the combination of the subcutaneously given small molecule **miridesap** (CPHPC) and the intravenously applicated SAP-specific antibody **dezamizumab** was studied. Miridesap was given in order to prevent the formation of circulating immune complexes by enhancing the hepatic clearance of circulating SAP (Richards et al., 2015). Because of a "change in benefit/risk profile", however, the study was halted (data not yet published).

4 | DISCUSSION AND CONCLUSION

ATTRv-amyloidosis has become a model disease for pathophysiology-based novel treatment approaches (Adams et al., 2019). To prevent amyloid formation, TTR can either be stabilized or knocked down, and amyloid-derived antibodies have been investigated for deposit removal. As none of the yet approved drugs or those in clinical trials directly target the specific *TTR* mutation, they are all promising approaches for various phenotypes (leading neuropathy or cardiomyopathy) or onset types (early and late), including ATTRwt amyloidosis.

In order to understand each substance's benefits, accurate biomarkers of progression and treatment response are crucial. In the previous studies, compiled scores of neuropathy severity, including, for example the NIS, NIS-LL, mNIS+7, PND, COMPASS-31, and Norfolk QoL were used as primary and secondary outcome parameters for neuropathy, whereas overall and cardiovascular mortality, 6-minute walking test, left ventricular wall thickness, global longitudinal strain, (NT-pro)BNP, and other values were used to measure progression of cardiomyopathy (Table 1). Because of limitations such as subjectivity of such scores or complexity in assessment, the search for new markers will have to go on in the future. One promising

approach to objectively detect axonal damage is, for example, measuring neurofilament light chain (NFL) serum levels, which is not specific for ATTR-related neuropathies, but seems to correlate with the clinical course (Kapoor et al., 2019; Louwsma et al., 2020).

Between the three already approved drugs, tafamidis, patisiran, and inotersen, no head-to-head comparison has been made. Differences in the study designs including patient collectives and numbers, study duration, and outcome parameters do not allow to designate "the best" of all substances (Magrinelli et al., 2020). In the ongoing phase 3 trial on AG10, a third study arm with tafamidis-treated patients would have been interesting to compare the novel stabilizer directly with the current standard of care. For all already and yet to be approved drugs, real-life long-term data are required to define individual predictors for a good treatment response, and immersing investigation needed to fill the holes in pathophysiological understanding.

In contrast to treatment approaches for other neurodegenerative diseases, ATTR amyloidosis harbors the opportunity of targeting the liver as the main production site of the amyloidogenic protein while not being directly affected by the disease itself. To decrease the treatment burden associated with unpleasant application types or side effects, further drug modifications for a more specific hepatic uptake are the next steps under investigation. For the remaining 10% of TTR that is produced in the choroid plexus and retina, the blood–brain and blood–eye barrier needs to be overcome. Despite enthralling success in these past few years, TTR has therefore saved some demanding challenges for the future.

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CONFLICTS OF INTEREST

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