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REVIEW

Emerging therapies in transthyretin amyloidosis – a new wave of hope after years of stagnancy?

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Transthyretin amyloidosis (ATTR) is a rare, yet underdiagnosed disease characterized by progressive impairment of neurologic and cardiac function due to deposition of misfolded transthyretin. Despite great efforts, such as the introduction of orthotopic liver transplant, the devastating prognosis for both variant and wild-type ATTR patients remained unchanged over the last decades, mainly due to a lack of specific therapies. Fortunately, recent years saw the introduction of promising targeted therapies, which aim to interfere with the deposition of misfolded transthyretin (TTR) at various stages of the cascade underlying ATTR progression. These include TTR tetramer stabilizers (tafamidis, diflunisal, epigallocatechin-3-gallate), TTR silencers (inotersen, patisiran) and fibril disruptors (monoclonal antibodies, doxycycline and tauroursodeoxycholic acid). In the context of this review we explain their mechanisms of action, analyse their efficacy on neurologic and cardiac function based on all clinical trials conducted to date and discuss their clinical applicability. Eventually suggestions for future clinical research into the field are provided.

Keywords Transthyretin amyloidosis • Familial amyloid polyneuropathy • Tafamidis • Patisiran • Inotersen

Introduction

Amyloidosis refers to a clinically variable group of conformational diseases, in which amyloidogenic proteins aggregate to form insoluble, toxic ß-sheet fibrillar amyloid deposits in the extracellular space of various tissues, leading to organ dysfunction.[1] The specific type of amyloidosis is categorically named after its aetiological precursor. To date 36 precursor proteins are known, each of which expresses distinct organ tropism and clinical phenotype.[2]

This review will focus on transthyretin amyloidosis (ATTR), which includes two sub-types – wild-type (ATTRwt) and variant ATTR (ATTRv) – that vary regarding their pathogenesis. Their common precursor protein transthyretin (TTR) physiologically functions as a transport protein for thyroxin and retinol-binding protein. TTR is predominantly synthesized in the liver and occurs as a tetramer in its natural form.[3]

Variant ATTR, formerly known as hereditary/mutant ATTR, is an autosomal-dominant disorder.[2] More than 100 mutations in the TTR gene have been identified, the most frequent being a V30M variant, which lead to single amino acid substitutions.[4] These induce subtle alterations in the structure of wild-type TTR (wtTTR) and turn it into significantly less stable variant TTR (vTTR) with amyloidogenic properties. As a result, the natural tetramer tends to dissociate into monomers, which misfold and self-aggregate to form pathogenic fibrils.[5] While the exact clinical phenotype depends on the underlying mutation, cardiomyopathy, peripheral polyneuropathy and autonomic neuropathy with orthostatic hypotension and gastrointestinal dysautonomia are common.[6] Median survival is approximately 120 months.[7]

Wild-type ATTR in contrast does not feature specific genetic mutations. Instead it is assumed that with increasing age, wtTTR with physiological primary structure expresses an intrinsic propensity to dissociate into monomers and aggregate to form amyloidogenic fibrils.[8,9] Patients exhibit isolated, strong cardiac involvement, which may be preceded by carpal-tunnel syndrome. They typically present with complaints of dyspnoea and peripheral oedema due to diastolic heart failure or palpitations in the setting of atrial fibrillation.[6,10] The manifestation of neurologic symptoms,

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such as tingling sensations or mild numbness in the lower extremities, is rare in comparison with ATTRv. However, diagnosis of stage 1 polyneuropathy, detectable only by thorough neurologic clinical examination and electromyography, is crucial in ATTRwt patients because it provides the indication for specific treatment, as outlined below. Median survival is approximately 75 months.[6]

Whereas until recently therapeutic options in ATTRwt were limited to symptomatic relief, orthotopic liver transplant represented an option for specific treatment in ATTRv patients, aiming to replace the main source of vTTR with a donor organ that produces wtTTR.[11] While orthotopic liver transplant substantially reduces serum vTTR and increases survival, especially in early-onset V30M patients, pre-transplant symptoms typically remain and ATTR-associated cardiomyopathy, the main reason for mortality, frequently progresses.[11,12]

Thus, the need for less invasive, potent therapeutic options is evident. Recently, a number of new pharmaceuticals were developed, which promise to suppress the production of potentially amyloidogenic wt- and vTTR, as well as the consecutive fibril formation at different stages of the pathogenetic process. This review aims to explain their respective mechanism of action (*Figure 1*), evaluate their clinical efficacy and applicability based on recent clinical trials, and eventually formulate suggestions for future clinical research.

Current pharmaceutical approaches in the treatment of ATTR (*Table 1*)

Transthyretin tetramer stabilizers

Transthyretin tetramer stabilizers are small molecules that influence the rate-limiting step in the formation of amyloid fibrils, the dissociation of TTR tetramers into amyloidogenic monomers.[13] In 1996 it was first shown that binding of a ligand to either of the binding sites for thyroxin or retinol-binding protein on TTR stabilizes its tetrameric structure and reliably prevents TTR dissociation.[14] This principle, called 'native state kinetic stabilization', is utilized by modern TTR tetramer stabilizers.[15] Since then, several stabilizing compounds that predominantly bind to the mostly unoccupied (>95%) thyroxin binding sites were found, the most extensively researched being diflunisal and tafamidis.[16]

Diflunisal is a non-steroidal anti-inflammatory drug that achieves high serum concentrations when administered orally.[17] Typical indications include pain and chronic inflammatory diseases (i.e. osteoarthritis; rheumatoid arthritis). Diflunisal is not specifically approved for ATTR treatment by regulatory authorities of any country but can be used 'off-label'. At doses of 250 mg twice daily, as recommended in most studies, diflunisal induces complete kinetic stabilization of both wt- and vTTR, which results in adequate inhibition of fibril formation.[18,19] Despite that, fear of gastrointestinal, renal and cardiac side effects associated with chronic use of non-steroidal anti-inflammatory drugs limit the routine use of diflunisal in already vulnerable ATTR patients, especially those with cardiomyopathy.[20–22]

Tafamidis is approved by the European Medicines Agency for stage 1 polyneuropathy (oral, 20 mg once daily) and the U.S. Food and Drug Administration for cardiomyopathy (oral, 80 mg/61mg once daily) in adults with both ATTRv and -wt.[23,24]

Tafamidis is a small molecule that belongs to the group of benzoxazole carboxylic acids, which achieve high oral bioavailability, while lacking unfavourable non-steroidal anti-inflammatory drug activity.[25] Through binding to the thyroxin binding site on TTR with high affinity and selectivity, tafamidis induces dose-dependent kinetic stabilization of wtTTR and a range of vTTR variants (i.e. V30M, V122I, etc.) *in vitro*.[26] These results are supported by several clinical trials, which reported TTR stabilization in 89–100% of patients at various time points.[27–32]

Maximum plasma concentration is achieved at a median time of 2 h, which may be prolonged by simultaneous intake of food, whilst the extent of absorption remains unaffected. Continuous administration of tafamidis 20 mg once daily induces a steady state after 14 days. In the plasma tafamidis is highly protein-bound (99.9%) and metabolized by glucuronidation before slowly (half-life of 59 h) being eliminated via the bile.[23]

Epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea, belongs to the group of natural polyphenols, which have been identified as alternative compounds inducing native state kinetic stabilization. Among these, EGCG appears to be the most potent while exhibiting high tolerability and low toxicity.[33] EGCG stabilizes the tetrameric structure of both wt- and vTTR, preventing aggregation and fibril formation *in vitro*, *ex vivo* and *in vivo* (in ATTRv transgenic mice).[34,35] In addition, EGCG showed potential to disaggregate amyloid deposits and disrupt preformed fibrils, converting them into unstructured off-pathway oligomers.[34–36] Interestingly, EGCG elicits these effects through binding to a distinct EGCG binding site, ruling out concerns that it may compete for the thyroxin binding site with other TTR tetramer stabilizers.[37] In fact, *in-vitro* studies indicated that co-treatment with EGCG and a small molecule occupying the thyroxin binding site (e.g. tafamidis) resulted in more effective stabilization of ATTRv and -wt than treatment with either of the two alone.[37] *In vivo*, however, the synergistic effect of co-treatment with EGCG and tafamidis (for example) may be restrained, as EGCG interacts with human serum albumin, which is crucial for the transport and bioavailability of tafamidis.[23,38] The same applies to co-treatment with EGCG and inotersen.[39] Presumably due to its independent binding site, EGCG also mitigated amyloidogenesis in TTR variants that appeared refractory to TTR tetramer stabilizers binding the thyroxin binding site.[37]

Preliminary clinical data ($n = 14$) from ATTRv and -wt patients with cardiomyopathy (53% vs. 47%), consuming approximately 550 mg EGCG daily for 12 months, showed significant reductions in interventricular septal thickness and left ventricular myocardial mass.[40] Consecutively, it was reported that those reductions were most likely due to a reduction of amyloid load in the myocardium, indicating a potential clinical benefit of EGCG.[41]

Therapeutic antisense oligonucleotides

Oligonucleotides are short (13–25 nucleotides), single-stranded DNA molecules which, following the principle of Watson–Crick

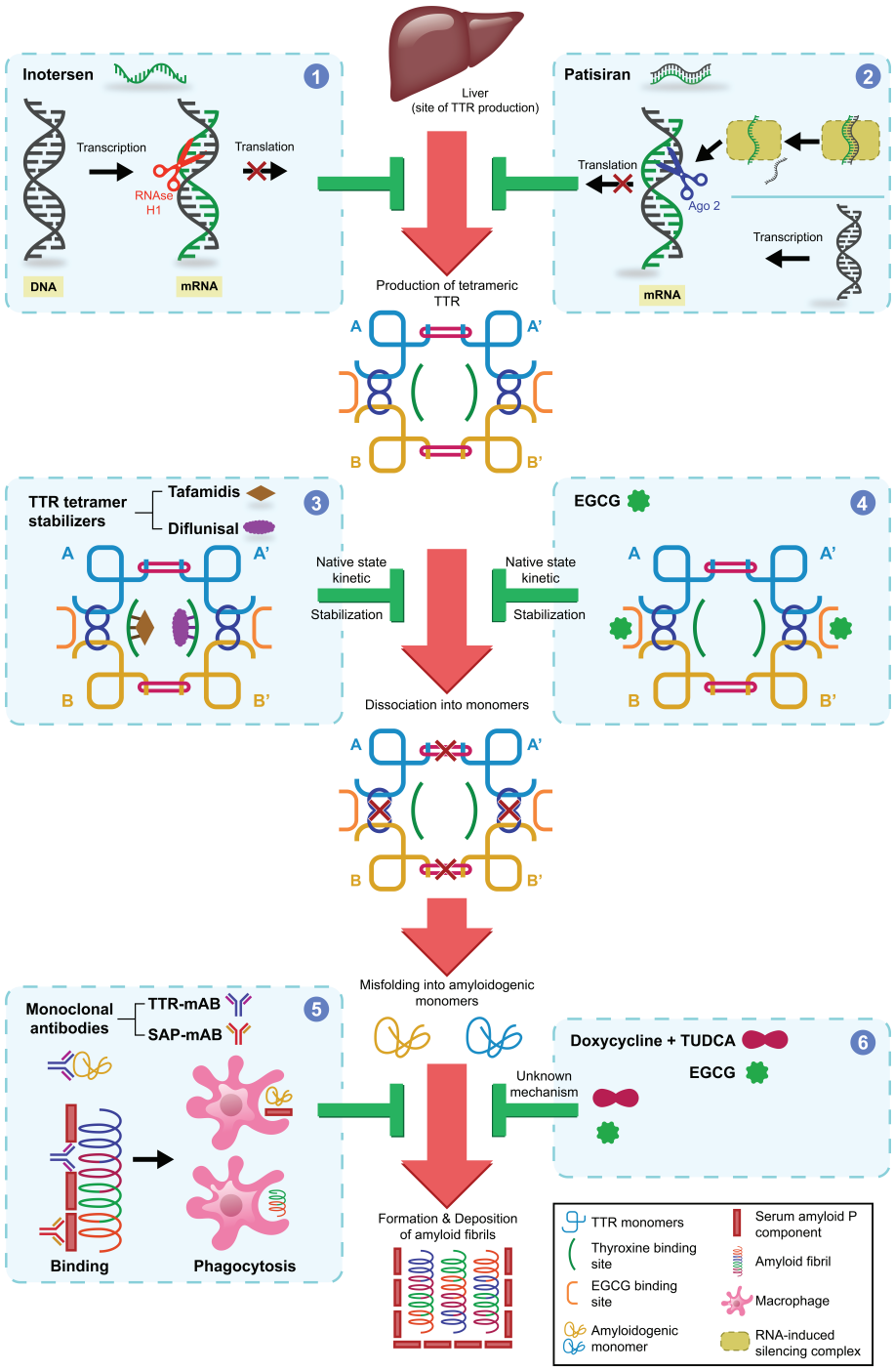

Figure 1 Pharmaceuticals in the treatment of transthyretin amyloidosis (ATTR) interfere at different stages of the transthyretin (TTR) amyloid cascade. (*1*) Inotersen attaches to TTR mRNA directly, inducing cleavage of the latter by the endonuclease RNase-H1, which prevents translation and hence reduces TTR production. (*2*) Upon binding to the RNA-induced silencing complex (RISC), patisiran loses its inactive sense strand. The pharmacologically active antisense strand attaches to TTR mRNA and induces cleavage by the endonuclease Ago2, which results in the prevention of translation and a reduced TTR production. (*3*) The TTR tetramer stabilizers tafamidis and diflunisal bind to the thyroxine binding site on tetrameric TTR and inhibit its dissociation into amyloidogenic monomers by native state kinetic stabilization. (*4*) Epigallocatechin-3-gallate (EGCG) induces a similar effect through binding to a distinct EGCG binding site. (*5*) Monoclonal antibodies against serum amyloid P component (SAP) and TTR (bind to both misfolded, pre-fibrillar TTR and fibrillar TTR deposits) attach to their specific target and induce phagocytic clearance of the latter by macrophages. (*6*) EGCG and the combination of doxycycline and tauroursodeoxycholic acid (TUDCA) disrupt fibrillar TTR deposits through an unknown mechanism.

Table 1 Overview of pharmaceuticals used for the treatment of transthyretin amyloidosis (status as of November 2019)

Pharmaceutical (trade name)	Mechanism	Approval		
		By	Indication	In
Tafamidis (Vyndaqel)	TTR tetramer stabilizer	EMA	Polyneuropathy (Stage I)	ATTRv & -wt
		FDA	Cardiomyopathy	ATTRv & -wt
Inotersen (Tegsedi)	TTR silencer	EMA	Polyneuropathy (Stage I and II)	ATTRv
		FDA	Polyneuropathy (any Stage)	ATTRv
Patisiran (Onpattro)	TTR silencer	EMA	Polyneuropathy (Stage I and II)	ATTRv
		FDA	Polyneuropathy (any Stage)	ATTRv
		Current use		
Diflunisal	TTR tetramer stabilizer	Off-label		
EGCG	TTR tetramer stabilizer; Fibril disruptor	Natural compound in green tea		
Doxycycline and TUDCA	Fibril disruptors	Off-label		
Monoclonal antibodies	Fibril disruptors	Pre-clinical (phase 1)		

ATTRv, variant transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; EGCG, epigallocatechin-3-gallate; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; TTR, transthyretin; TUDCA, tauroursodeoxycholic acid.

base pairing, fit exactly one specific sequence in the genetic pool of a cell. In an attempt to overcome problems concerning the stability of antisense oligonucleotides (ASOs) and the toxicity of their degradation products, most ASOs are chemically modified.[42] They act as antisense strands to specific mRNA sequences coding for pathogenic proteins (i.e. wt- and vTTR) and regulate the expression of that mRNA.[43] Upon binding to the target mRNA, ASOs can employ two distinct regulatory mechanisms, depending on their molecular design. These are (i) steric blocking, in which ASOs prevent translation by plain occupancy of the mRNA, and (ii) enzymatic mechanisms inducing RNA cleavage or degradation.[44]

Inotersen (subcutaneous, 284 mg once a week) is an ASO inhibitor of hepatic TTR production, approved by the European Medicines Agency (only stage 1 and 2 polyneuropathy) and the U.S. Food and Drug Administration for the treatment of polyneuropathy in adult patients with ATTRv.[39,45]

Inotersen features a 20 nucleotide gapmer design with 10 central DNA nucleotides that sustain the RNase mechanism. The 5′- and 3′-ends contain 5 nucleotides each, which are chemically modified by replacing hydrogen at the 2′-position of ribose with an O-methyl group (i.e. 2´-O-methoxyethyl-modified ASO).[39,44,45] These modifications improve binding affinity to target mRNA, provide stability against nucleases and reduce unfavourable pro-inflammatory properties.[44]

Inotersen binds to wt- or vTTR mRNA forming high melting DNA–RNA heteroduplexes, which activate an RNase H1-dependent mechanism.[44,46,47] Specifically, the endonuclease RNase H1 induces cleavage of wt- and vTTR mRNA, leading to its degradation, which prevents synthesis of both forms of TTR in the liver.[48,49]

In-vitro experiments on patient-derived hepatocyte-like cell lines showed dose-dependent down-regulation of TTR mRNA of more than 80% and the clinical NEURO-TTR study reported a mean reduction in serum TTR of 74%, regardless of mutation type.[50,51] Higher doses (i.e. 400 mg) did not show superior effects.[47] Due to its function as a transport protein for retinol-binding protein, reductions in serum TTR were accompanied by a reduction in serum vitamin A (−63%). Therefore, supplementation of vitamin A (3000 IU daily) is recommended for patients treated with inotersen.[51]

Pharmacokinetics of inotersen are dose-dependent. Maximum plasma concentration is achieved at a median time of 2–4 h and steady state is reached after approximately 3 months of treatment. More than 94% of inotersen is bound to plasma proteins, supporting its broad distribution to tissues.[39] The main way of elimination is metabolization by endonucleases, which split inotersen into nucleotides of variable length. The half-life is approximately 32 days.[39]

Small interfering RNA

Technically, small interfering RNA (siRNA) can be counted among the group of ASOs. The main difference to single-stranded ASOs is that siRNA consists of double-stranded RNA oligonucleotides. Similar to ASOs the antisense strand is pharmacologically active, whereas the additional sense strand in siRNA serves as a vehicle for transport into the cytoplasm and protects the antisense strand from degradation.[52] Initial problems regarding the ability of hydrophilic siRNA to overcome lipid bilayers and deliver RNA into cells (i.e. hepatocytes) have recently been resolved by (i) formulation of siRNA into cationic lipids/nanoparticles, or (ii) chemical modifications of siRNA to interact with high-capacity cell surface receptors.[53] Upon arrival in the cytoplasm the siRNA double strand binds to Ago2 in the RNA-induced silencing complex and releases the inactive sense strand. Ago2 functions as an endonuclease that cleaves RNA in RNA–RNA duplexes.[54] The remaining Ago2 antisense complex then hybridizes to the target mRNA (i.e.

wt–/vTTR mRNA) and induces cleavage and catalytic degradation of the latter.[55]

Patisiran (intravenous, 0,3 mg/kg every 3 weeks) is an siRNA drug approved by the European Medicines Agency (only stage 1 and 2 polyneuropathy) and the U.S. Food and Drug Administration for the treatment of polyneuropathy in adult patients with ATTRv.[56,57]

Due to the risk of infusion-related reactions, patients need to receive premedication including dexamethasone, oral acetaminophen/paracetamol, an H_2-blocker and an H_1-blocker.[56]

Patisiran is made up of two RNA strands, each of which contains 21 nucleotides. Chemical modifications include 11 nucleotides that feature 2′-methoxy sugar residues and 4 nucleotides with 2′-deoxy thymidine residues.[57] The formulation of patisiran into lipid nanoparticles facilitates specific delivery to the primary site of TTR production, the hepatocytes in the liver.[56,58]

The pharmacologically active antisense strand targets a highly conserved genetic sequence in the 3′-untranslated region of wt- and vTTR mRNA, suppressing production of both TTR types by the Ago2-mediated mechanism described above.[56] In fact, no known TTR variant interferes with that target sequence, promising consistent efficacy across all ATTR patients, regardless of TTR status and mutation type.[59,60]

A single dose of patisiran showed >85% reduction of hepatic TTR mRNA and serum TTR in murine models.[60] Biweekly administration induced TTR knockdown of >95% and led to statistically significant reduction of TTR deposits in various tissues, with some animals achieving nearly complete TTR deposit reduction.[60]

A phase 2 multi-dose study ($n = 26$) on ATTRv patients indicated a dose-dependent effect of patisiran, with maximum reductions in serum TTR of 87% at doses of 0.3 mg/kg administered every 3 weeks.[61] The subsequent clinical APOLLO-II trial ($n = 225$) reported congruent results with mean reductions in serum TTR of 81% over a period of 18 months, regardless of mutation type.[62] Equivalent to inotersen, the reduction in serum TTR correlated with reductions in retinol-binding protein and serum vitamin A.[61] Hence, supplementation of vitamin A (3000 IU daily) is recommended.[56]

Patisiran exhibits dose-dependent pharmacokinetics, with higher doses leading to higher systemic exposure. A steady state is reached after 24 weeks of treatment, with slight accumulation.[56] The majority of patisiran distributes straight to the liver and less than 2% is bound to plasma proteins. Metabolization by nucleases is the main way of elimination, leading to a half-life of approximately 76 h.[56]

Doxycycline and tauroursodeoxycholic acid

Doxycycline and ***tauroursodeoxycholic acid*** are not specifically approved for treatment of ATTR but appear to be a promising combination, capable of disrupting TTR fibrils in existing amyloid deposits of ATTRv and -wt patients. Doxycycline is a derivative of tetracycline, an antibiotic that possesses anti-amyloidogenic properties.[63] Among several tetracycline derivatives tested *in vitro*, doxycycline proved to be the most effective in disrupting preformed TTR fibrils, whilst its effect on non-/pre-fibrillar TTR deposits was insufficient.[63] These findings were confirmed in V30M transgenic mice.[64] Interestingly, tauroursodeoxycholic acid, a biliary acid, was studied in similar transgenic mice and induced a significant reduction of non-/pre-fibrillar TTR deposits exclusively.[65] Eventually, the combination of doxycycline and tauroursodeoxycholic acid was evaluated and showed strong synergistic effects in the destruction and reabsorption of TTR deposits.[66] Ursodeoxycholic acid is a bile acid with an efficacy similar to that of tauroursodeoxycholic acid and has been used as an alternative in some studies.[67] Chemical modifications, such as polymer-conjugation of doxycycline, even bear the potential for further improvements of those effects.[68]

A phase 2 study ($n = 20$) on ATTRv and -wt patients with daily administration of oral doxycycline (100 mg) and tauroursodeoxycholic acid (3×250 mg), reported stable disease over the duration of 12 months.[69]

Monoclonal antibodies

Monoclonal antibodies are the latest addition to the growing list of potential treatments for ATTR. While using similar concepts, two subgroups [anti-TTR; anti-serum amyloid P component (anti-SAP)] of monoclonal antibodies are known that vary regarding their specific target.

Anti-TTR antibodies were developed following a report claiming that the native immune system produces catalytic IgM class antibodies specific to misfolded TTR in healthy subjects.[70] During the pathogenic formation of ß-sheet fibrils, conformational changes expose cryptic epitopes on the molecular surface of amyloidogenic TTR, which serve as drug targets.[71,72] That allows anti-TTR antibodies to attach to amyloid fibrils and pre-fibrillar TTR of both types with high affinity and specificity, while sparing the physiological TTR tetramer lacking these epitopes.[73] Binding of the monoclonal antibody inhibits TTR fibrillization in a dose-dependent manner, preventing the formation of new amyloid deposits, and promotes phagocytosis of aggregated TTR by macrophages, leading to tissue clearance.[74–76] A variety of monoclonal antibodies against several TTR epitopes has been developed and tested for their specificity and efficacy *in vitro*.[72–77] However, to the best of our knowledge, none of them have been tested *in vivo* and were identified to be suitable for administration to humans.

Dezamizumab (anti-SAP) is an antibody (IgG1) that targets SAP, a non-fibrillar plasma glycoprotein ubiquitously present in all types of human amyloid deposits.[78] It is administered after depletion of circulating serum SAP by *miridesap*/CPHPC, which allows dezamizumab to specifically target and degrade SAP-containing amyloid deposits via complement activation and a macrophage-dependent phagocytic clearance mechanism, similar to the one described above.[78,79] Following promising results in murine models, an open-label multi-dose phase 1 trial, administering serial doses of dezamizumab, was conducted on 38 patients with systemic amyloidosis, three of which suffered from ATTR.[78,80,81] Herein, dezamizumab reduced amyloid deposits in the kidney (especially at

Table 2 Overview of all studies analysing the effect of various pharmaceuticals on transthyretin-associated amyloid polyneuropathy (status as of June 2019)

Author	Patients		Application		Primary outcomes
	n	TTR status (ATTRv /—wt)	Dose	Time (months)	
Tafamidis					
Coelho *et al.*[27]	128	ATTRv (V30M only)	20 mg once daily	18	NIS-LL Norfolk QoL-DN
Coelho *et al.*[28]	85	ATTRv (V30M only)	20 mg once daily	12 (extension)	NIS-LL Norfolk QoL-DN
Merlini *et al.*[32]	21	ATTRv (non-V30M)	20 mg once daily	12	TTR stabilization (week 6)
Barroso *et al.*[82]	93	ATTRv (all mutations)	20 mg once daily	42 (extension)	NIS-LL Norfolk QoL-DN
Lozeron *et al.*[31]	36	Advanced ATTRv (V30M only)	20 mg once daily	30	NIS-LL/UL
Cortese *et al.*[83]	61	ATTRv (all mutations)	20 mg once daily	36	Not defined, but outcomes included NIS and NIS-LL
Planté-Bordeneuve *et al.*[84]	43	ATTRv (all mutations)	20 mg once daily	24	NIS
Diflunisal					
Berk *et al.*[18]	130	ATTRv (all mutations)	250 mg twice daily	24	NIS+7
Sekijima *et al.*[85]	40	ATTRv (all mutations)	250 mg twice daily	38 (mean)	Not defined
Takahashi *et al.*[86]	6	ATTRv (V30M only)	250 mg twice daily	53 (mean)	Not defined
Inotersen					
Benson *et al.*[51] (NEURO-TTR)	172	ATTRv (all mutations)	300 mg once a week	15	mNIS+7 Norfolk QoL-DN
Patisiran					
Adams *et al.*[62] (APOLLO)	225	ATTRv (all mutations)	0.3 mg/kg bodyweight every 3 weeks	18	mNIS+7

ATTRv, variant transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; NIS-LL/UL, Neuropathy Impairment Score in the Lower Limbs/Upper Limbs; QoL-DN, Quality of Life Diabetic-Neuropathy; TTR, transthyretin; (m)NIS+7, (modified) neuropathy impairment score +7.

>1200 mg) and liver (especially at >2000 mg), as assessed by SAP scintigraphy and liver stiffness, in a dose-dependent fashion.[81] An effect on cardiac amyloid deposits could not be shown, although transient increases in N-terminal pro-brain natriuretic peptide (NT-proBNP) were interpreted as the initiation of a mechanism for potential cardiac amyloid clearance.[81]

Clinical efficacy – what do we know so far?

In this section we intend to provide a comprehensive overview of the clinical trials undertaken on each of the pharmaceuticals described above and evaluate their results. If pharmaceuticals are not discussed in this section, no valid clinical data are available at this point to the best of our knowledge. A summary of all trials taken into consideration can be found in *Table 2* (polyneuropathy)[18,27,28,31,32,51,62,82–86] and *Table 3* (cardiomyopathy).[29,30,67,87–89]

Findings concerning ATTR-associated polyneuropathy

Tafamidis has been evaluated in a total of seven studies with primary endpoints concerning polyneuropathy. Due to a lack of validated outcome measures for ATTR-associated polyneuropathy in particular, all studies used scores with previously demonstrated validity in diabetic polyneuropathy.[90,91] These included the response to treatment (<2 points increase) of the Neuropathy Impairment Score in the Lower Limbs/Upper Limbs (NIS-LL/-UL), which assess the loss of motoric, sensory and reflex functions, as well as the least-squares mean change in the Norfolk Quality of Life Diabetic-Neuropathy Questionnaire (Norfolk QoL-DN), providing a subjective total quality of life score. Since then, the Norfolk QoL-DN has been validated for trials on ATTRv patients with polyneuropathy and it was shown that the NIS-LL correlates with disease stage.[92,93]

Coelho and colleagues first published data on 128 ATTRv patients with V30M mutations, who received tafamidis 20 mg

Table 3 Overview of all studies analysing the effect of various pharmaceuticals on transthyretin-associated amyloid cardiomyopathy (status as of June 2019)

Author	Patients		Application		Primary outcomes
	n	TTR status	Dose	Time (months)	
Tafamidis					
Damy *et al.*[87]	21	ATTRv (non-V30M)	20 mg once daily	12	TTR stabilization (week 6)
Maurer *et al.*[29]	35	ATTRwt (89%) & ATTRv (11%)	20 mg once daily	12	TTR stabilization (week 6)
Maurer *et al.*[30] (ATTR-ACT)	441	ATTRwt and ATTRv	20 mg or 80 mg once daily	30	All-cause mortality Cardiovascular-related hospitalizations
Diflunisal					
Castaño *et al.*[88]	13	ATTRv and ATTRwt	250 mg twice daily	11 (mean)	Not defined (exploratory cardiac outcomes)
Patisiran					
Solomon *et al.*[89] (APOLLO)	126 (cardiac subpopulation)	ATTRv (all mutations)	0.3 mg/kg bodyweight every 3 weeks	18	mNIS+7
Doxycycline and TUDCA					
Karlstedt *et al.*[67]	53	ATTRwt (89%) & ATTRv (11%)	Doxycycline 100 mg (2x/d) and ursodeoxycholic acid 250 mg (3x/d)	22 (median)	None (retrospective analysis of clinical data)

ATTRv; variant transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; TTR, transthyretin; TUDCA, tauroursodeoxycholic acid; mNIS+7, modified neuropathy impairment score +7.

($n = 65$) once daily for 18 months.[27] Whilst they could not show significant differences in the intention-to-treat population, analysis of the efficacy-evaluable population showed significantly more NIS-LL response (60.0% vs. 38.1%, $P = 0.041$) and less change in the Norfolk QoL-DN (0.1 vs. 8.9 points, $P = 0.045$), suggesting a decelerated deterioration of neurological function that led to preserved quality of life in patients treated with tafamidis. In particular, tafamidis prevented muscle weakness, especially at distal joints, reduced the loss of small nerve fibre function and improved nutritional status, as measured by modified body mass index.[27]

These results could be replicated in an open-label extension study, in which all patients received tafamidis 20 mg for 12 months ($n = 85$), indicating sustained efficacy of tafamidis over 30 months.[28] Interestingly, patients that only started to receive tafamidis in the extension period showed similar results for the Norfolk QoL-DN and nutritional status to those who received tafamidis for 30 months, indicating that aggravation of those factors may be reversible under tafamidis treatment. However, neurological disease progression resulting from a lack of treatment for the first 12 months could not be reversed by the initiation of treatment, resulting in a significant difference in the mean change for NIS-LL between the two groups (3.0 vs. 6.8 points, $P = 0.04$).[28] Thus, early initiation of treatment appears to be essential.

The interim analysis of an ongoing open-label extension (10 years; $n = 93$) of the two studies discussed above and a single-arm open-label study in non-V30M patients discussed below reinforced these results in V30M patients for a treatment duration of up to 6 years.[82]

Unfortunately however, non-V30M ATTRv patients ($n = 18$) progressed in respect to neurologic dysfunction (least-squares mean change in NIS-LL of 14.2 points in 48 months) and quality of life (least-squares mean change in Norfolk QoL-DN of 24.8 points in 48 months), despite being treated with tafamidis.[82] Preceding these findings, Merlini and colleagues published congruent results for a variation of non-V30M mutations from a phase 2 open-label study ($n = 21$).[32] Based on equal results, they concluded that tafamidis elicits similar effects across different non-V30M mutations. However, in light of the small number of participants, this conclusion appears speculative. Additionally, they found that neurological disease progression was more severe in patients with higher baseline values of NIS (i.e. more advanced disease stages), substantiating the need for early treatment initiation.[32]

This appears to equally be the case for V30M patients. Lozeron and colleagues conducted a study ($n = 37$) focused on significantly more advanced late-onset cases of V30M ATTRv (mean NIS-LL at baseline: 27.2), which showed that tafamidis could not prevent disease progression in those patients.[31] In fact, the mean increase of NIS-LL (+4.8) in on-treatment patients was close to pre-treatment values (+3.2) and only two patients remained stable with respect to disability, as measured by three disability scores.[31] These findings were later confirmed for both V30M and non-V30M patients by

Cortese *et al.*[83] ($n = 61$) and Planté-Bordeneuve *et al.*[84] ($n = 43$), who reported that late disease-onset and advanced disease at baseline are predictors of inferior response to tafamidis.[83,84] Additionally, Planté-Bordeneuve and colleagues confirmed that carriers of non-V30M mutations are significantly less likely to respond to treatment with tafamidis, as discussed above.[84]

Diflunisal was first assessed in ATTR-associated polyneuropathy by Berk and colleagues in a randomized, placebo-controlled study on ATTRv patients ($n = 130$; 54.6% V30M).[18] Patients received diflunisal 250 mg twice daily ($n = 64$) or placebo ($n = 66$) for 24 months. The primary endpoint was the difference in mean change of the Neuropathy Impairment Score + 7 (NIS+7) between the two groups at 24 months. Although it was also only validated for diabetic neuropathy, the NIS+7 constitutes a composite score that adds the assessment of seven neurophysiologic test results to the conventional NIS-LL used in tafamidis trials.[90]

Berk and colleagues reported a significant difference (18.1 points; $P < 0.001$) in mean change of NIS+7 between placebo (+26.3 points) and diflunisal (+8.2 points) along with concordant changes in NIS, NIS-LL and clinical Kumamoto score, indicating that diflunisal mitigates neurological disease progression in ATTRv.[18] Interestingly, these changes were already observable at 12 months and irrespective of mutation type or neuropathy stage at baseline. Additionally, physical and mental quality of life could be stabilized in patients taking diflunisal. Following the study by Coelho and her team on tafamidis,[27] this study provides further clinical proof of the concept of 'native state kinetic stabilization'. In contrast to tafamidis, however, an improvement of nutritional status was not observable.[18]

A subsequent study by Sekijima and colleagues administering diflunisal 250 mg twice daily to Japanese patients with ATTRv ($n = 40$; 75% V30M) for an average of 38 months, confirmed these results.[85] Although diflunisal could not stop disease progression, it was significantly slowed when compared to natural history of ATTR, as indicated by mean annual change in Kumamoto score (+1.0/year vs. +3.3−7.0/year). Furthermore, it could be shown that beneficial effects of diflunisal are sustained after 24 months of treatment. Symptoms of autonomic dysfunction, such as nausea and diarrhoea or constipation, improved in approximately 30% of patients, while they worsened in the remaining 70%.[85] Similar observations were made by Takahashi and colleagues in a small group of Japanese late-onset ATTRv patients with V30M mutations ($n = 6$), where symptoms of autonomic dysfunction (including orthostatic hypotension) disappeared in 50% of patients within 1 month of treatment initiation.[86]

Inotersen is the subject of the recent NEURO-TTR study conducted by Benson and colleagues ($n = 172$).[51] In this randomized, double-blind, controlled trial, 112 ATTRv patients with 27 different mutations (50% V30M) received weekly subcutaneous injections of inotersen 300 mg for 15 months and were compared to 60 patients receiving placebo. All patients were pre-treated with vitamin A to compensate for reductions in serum vitamin A, discussed earlier. The co-primary endpoints were least-squares mean change in the modified NIS+7 (mNIS+7), an adapted version of the NIS+7 specifically designed for ATTR, and the Norfolk QoL-DN, both of which have been validated for use in therapeutic trials on ATTRv patients with polyneuropathy.[92,94,95]

Benson and colleagues reported that inotersen induced stable disease (no change from baseline) in 36% and 50% of ATTRv patients, considering the mNIS+7 and Norfolk QoL-DN, respectively. Although on average, inotersen could not completely halt disease progression in respect to mNIS+7 (+5.8 points) and Norfolk QoL-DN (+1.0 points) over the course of 15 months, disease progression was slowed significantly in comparison to the placebo group, with least-squares mean differences of −19.7 points for the mNIS+7 and − 11.7 points for the Norfolk QoL-DN. All beneficial effects of inotersen were observed irrespective of mutation type, disease severity, previous treatment and presence of cardiomyopathy.

Patisiran has been analysed for its clinical efficacy in the randomized, double-blind, placebo-controlled APOLLO trial (n = 225) recently published by Adams and colleagues.[62] 148 ATTRv patients, with 39 different mutations (38% V30M), received 0.3 mg/kg body weight of intravenous patisiran every 3 weeks for 18 months and were compared to the placebo group ($n = 77$). A cardiac subpopulation ($n = 126$) was pre-defined to enable valid statistical analysis of the effect of patisiran on cardiac manifestations. In contrast to the trials evaluating tafamidis and inotersen, the APOLLO trial focused on the mNIS+7 (change from baseline to 18 months) as the single primary endpoint. Among others, the Norfolk QoL-DN was included as a secondary endpoint.

Notably, patisiran induced an actual improvement (decrease from baseline) of the neurological impairment in 56% of patients over the course of 18 months, with a least-squares mean change of −6.0 points in mNIS+7 and least-squares mean difference of −34.0 points when compared with placebo. These beneficial effects were detectable as early as 9 months from treatment initiation and were significant for all subgroups. Whilst 65% of patisiran patients were stable regarding neuropathy stage, as assessed by the polyneuropathy disability score, 8% even improved, implying a transition from assisted to unassisted walking. Similar improvements were observed regarding quality of life, with a least-squares mean change in Norfolk QoL-DN of −6.7 points and a least-squares mean difference of −21.1 points in comparison with placebo. Furthermore, patients reported significant improvements in walking ability, nutritional status and activities of daily life.

Findings concerning ATTR-associated cardiomyopathy

Tafamidis is the most extensively researched pharmaceutical with regard to its effect on ATTR-associated cardiomyopathy. In total, results from two phase 2 studies and a major phase 3 study haven been reported to date.

At first, a population of an open-label phase 2 study by Merlini and his group ($n = 21$)[32] was re-assessed for cardiac findings by Damy and colleagues using exploratory efficacy outcomes.[87] 81% of patients, all of which had non-V30M and non-V122I ATTRv, were found to have amyloid deposition in the myocardium, as diagnosed by left ventricular wall thickness > 12 mm. On average, administration of tafamidis 20 mg daily for 12 months induced

stable disease with regard to cardiac biomarkers (NT-proBNP and troponin I), echocardiographic and electrocardiographic variables. However, 33% of patients were found to have signs of modest cardiac disease progression (i.e. increase in interventricular septal thickness > 2 mm), when analysed individually.[87]

Shortly after, Maurer and his team published a phase 2 study ($n = 35$), similarly using exploratory efficacy endpoints to assess the effect of tafamidis 20 mg daily for 12 months on progression of cardiomyopathy.[29] In contrast to the study by Damy and colleagues,[87] this study focused on ATTRwt patients ($n = 31$), with only a few expressing V122I mutations ($n = 4$). Moreover, all patients (100%) were diagnosed with cardiac amyloidosis based on endomyocardial biopsy or left ventricular wall thickness >12 mm in the context of a diagnosis of amyloidosis based on biopsy of other tissues. While treatment with tafamidis appeared to stabilize echocardiographic and electrocardiographic variables, troponin I increased by 0.037 ng/mL ($P < 0.05$) on average. Additionally, 48% of patients were found to have clinical signs indicative of disease progression such as new-onset atrial fibrillation or hospitalization for heart failure.[29]

Although both studies concluded that tafamidis could potentially mitigate cardiac disease progression, the lack of a control group and the small number of patients made the results relatively inconclusive.

In an attempt to change that, Maurer and colleagues initiated the ATTR-ACT trial ($n = 441$).[30] In this randomized, double-blind, placebo-controlled phase 3 study patients with biopsy-proven ATTRv (24%) or ATTRwt (76%) and clinical signs of cardiomyopathy were assigned to receive tafamidis 80 mg, tafamidis 20 mg, or placebo ($n = 177$) once daily for 30 months. Patients receiving either of the two doses of tafamidis were merged into a pooled tafamidis group ($n = 264$) for statistical analysis. Primary endpoints were all-cause mortality, followed by hospitalizations. Additional secondary endpoints included a 6-min walk test and total quality of life, as assessed by the Kansas City Cardiomyopathy Questionnaire.

Overall, the results of ATTR-ACT confirm that tafamidis has superior effects on the progression of cardiomyopathy in ATTR patients, when compared to placebo. The cumulative effect of tafamidis on all primary endpoints was assessed using the Finkelstein–Schoenfeld method, for which a win ratio of 1.695 was calculated, indicating that 69.5% of patients receiving tafamidis benefited from treatment in terms of a reduced risk of death or hospitalization over the course of 30 months. Furthermore, all-cause mortality (29.5% vs. 42.9%) and cardiovascular-related hospitalizations (0.48 vs. 0.70 hospitalizations/year) were reduced individually and irrespective of TTR subtype (ATTRv vs. -wt), when compared to placebo. However, differences in survival only reached significance after a treatment duration of 18 months. Additionally, it was found that patients with New York Heart Association (NYHA) class III (indicating more advanced disease at baseline) were the only ones not to benefit from tafamidis, specifically due to higher rates of hospitalization.[30] These findings stress the need for early treatment initiation and good compliance over a prolonged period of treatment.

Interestingly, no significant difference was found between the two tested doses (20 mg vs. 80 mg)[30], while *in-vitro* results suggested the need for doses of 60 mg daily to achieve complete kinetic stabilization, especially in more advanced cases.[96]

In addition to the improvement of primary endpoints, treatment with tafamidis also slowed the deterioration of walking ability and quality of life, which was detectable from as early as 6 months.[30]

Diflunisal's effect on ATTR-associated cardiomyopathy has only been evaluated in a small single-arm, open-label study by Castaño and colleagues.[88] All patients ($n = 13$; 54% ATTRwt, 46% ATTRv) had biopsy-proven cardiac amyloid with concordant clinical symptoms and received diflunisal 250 mg twice daily for an average of 12 months. At the end of the study period, no significant changes in left-ventricular mass index and ejection fraction were observable, indicating that diflunisal may be beneficial in maintaining cardiac structure and function. However, these results are preliminary and will need to be confirmed in larger systematic trials.

Inotersen has not been systematically examined for its effect on cardiomyopathy in ATTR patients. The NEURO-TTR study ($n = 172$) described above, showed no significant difference in echocardiographic variables between inotersen and placebo.[51] Preliminary results of an open-label study ($n = 22$), presented in an abstract by Dasgupta and colleagues, indicate stabilization of structural and functional cardiac parameters in the majority of both ATTRv and -wt patients.[97] , However, reliable evidence of inotersen's effect on cardiomyopathy in ATTR patients will have to be gathered in randomized, controlled trials, including higher numbers of patients.

Patisiran was primarily studied in the APOLLO trial ($n = 225$) described above.[62] In a recent publication, Solomon and colleagues analysed the data for exploratory endpoints in a pre-specified cardiac subpopulation ($n = 126$), including ATTRv patients with left ventricular wall thickness ≥13 mm and no history of aortic valve disease or hypertension.[89] Patients were previously classified as NYHA class I (40%) and NYHA class II (60%), whilst patients with higher classes were excluded. The exploratory endpoints comprised echocardiographic parameters, as well as cardiac biomarkers NT-proBNP and troponin I. Patisiran induced an average reduction in left ventricular wall thickness of −0.9 mm ($P = 0.017$) compared to baseline, along with reductions in interventricular septal thickness and left ventricular mass.[89] In consideration of the mostly unchanged values for patients receiving placebo, these results indicate that patisiran may lead to the reversal of structural changes in the myocardium, typically evoked by amyloid deposition. At the same time absolute improvement (<−2%) of global longitudinal strain, a measure of myocardial function, was traceable in only 21.3% of patients treated with patisiran. Compared to placebo, however, patisiran induced a relative reduction in global longitudinal strain (−1.4%, $P = 0.015$) and increased cardiac output by 0.38 L/min ($P = 0.044$).[89] Hence it seems like patisiran halts the aggravation of myocardial dysfunction in most patients, while actual improvement is limited to a few cases. NT-proBNP decreased in 31.6% and remained stable in 47.3% of patisiran patients, with a mean reduction of 55% compared to placebo. Post-hoc analysis of the safety data also showed a reduction in all-cause hospitalization

(−50%), cardiac hospitalization (−45%) and all-cause mortality (−45%).[62,89]

Doxycycline and ***ursodeoxycholic acid*** were evaluated for their efficacy in ATTR patients with cardiomyopathy in a retrospective analysis (n=53) without control group published by Karlstedt and colleagues.[67] Patients, predominantly suffering from ATTRwt (89% vs. 11% ATTRv), received doxycycline 100 mg twice daily and ursodeoxycholic acid 250 mg three times a day for a median follow-up of 22 months. In the context of routine care, clinical appointments were scheduled every 6 months and echocardiography, as well as tests for NT-proBNP and troponin T were performed every 12 months.

Interestingly, 38% of patients improved in regard to left ventricular global longitudinal strain (−12% to −17%; $P < 0.01$), suggesting enhanced mechanical function of the myocardium. These patients were generally younger and at earlier disease stages, as indicated by lower biomarker levels and NYHA stage at baseline. However, overall no significant changes in NT-proBNP, troponin T and echocardiographic variables were observed.[67] Whilst this might not seem like a favourable outcome at first, it implies that doxycycline and ursodeoxycholic acid also halt disease progression. This is particularly surprising given that 47% of patients had NYHA class III/IV at baseline[67], indicating advanced disease stages that were excluded in other studies, and considering that the underlying mechanism of action only promises destruction of pre-existing amyloid deposits. Thus, combinations with pharmaceuticals inhibiting the formation of new amyloid deposits could bear great potential.

Safety profiles

In consideration of the broad clinical presentation of ATTR patients with progressive dysfunction of diverse organs, especially at more advanced disease stages, possible adverse events should be considered as another variable in a clinician's attempt to elicit the best pharmaceutical option for the individual patient. This section summarizes the treatment-related adverse events reported to date. Events for which a connection to treatment could legitimately be ruled out are not included.

Tafamidis has the most comprehensively described safety profile due to the multitude of clinical trials available. The overall incidence of adverse events and serious adverse events was comparable to that of placebo groups in most trials and did not seem to increase with higher exposure time to tafamidis.[27,28] Hence, tafamidis was generally considered well-tolerated. The most common serious treatment-emergent adverse events were urinary tract infections (2−17%), some of which were described as febrile.[27,28,31,82] Especially after treatment initiation some patients also had severe diarrhoea with foecal incontinence, whilst others reported constipation.[31,82] Interestingly, most studies reporting higher incidences of these adverse events consisted of patients with primary symptoms of polyneuropathy. In contrast, results from 441 ATTR patients with cardiomyopathy actually showed lower rates of urinary tract infections and diarrhoea for patients receiving tafamidis, when compared to placebo.[30]

Other treatment-related adverse events included falls (and sporadic accounts of ataxia/syncope), pleural effusions and slight renal impairment.[29,82]

Cardiac events, such as transient ischaemic attacks, atrial fibrillation, cardiac failure, chest pain and dyspnoea, were considered part of the progressive nature of ATTR-associated cardiomyopathy and not related to tafamidis.82

Electrocardiographic results from Merlini and colleagues ($n = 21$) showed cumulative events of arrhythmia (+30.1% from baseline), particularly premature atrial contractions.[32] However, it was concluded that tafamidis has no adverse effect on cardiac conduction and repolarization. This was confirmed, considering the QT_C interval in particular, in a consecutive study ($n = 42$) by Klamerus and his team, administering supratherapeutic (400 mg) single doses of tafamidis to healthy volunteers.[98] In general, laboratory analyses did not show any differences between tafamidis and placebo.[27,30] No treatment-related deaths were reported.

Diflunisal's safety profile is of particular interest because chronic use of non-steroidal anti-inflammatory drugs has previously been linked to gastrointestinal injury, hypertension, renal dysfunction and worsening of heart failure.[20–22]

Placebo-controlled data on the safety of diflunisal are limited to the study by Berk and colleagues as discussed above.[18] While some side effects had been observed, the incidence of serious and drug-related adverse events among patients who had been treated with diflunisal was similar to the control group, indicating that diflunisal was generally well-tolerated.[18]

In particular, diflunisal induced gastrointestinal injury that led to bleeding in one patient (1.5%),[18] while it caused gastric intolerance in 13% of patients in another study.[99] However, the risk of gastrointestinal injury can be reduced by proton pump inhibitors or histamine type 2 receptor antagonists.[85,88]

Worsening of heart failure led to discontinuation of diflunisal in one patient (1.5%) in the study by Berk and colleagues, but was not encountered in other studies.[18,85] In regard to cardiac function, administration of diflunisal appeared to be safe overall, even in patients diagnosed with ATTR-associated cardiomyopathy.[88,99]

Additionally, some patients experienced a deterioration of renal function that aggravated over time and improved shortly after diflunisal was discontinued.[85,88,99] Hence, close renal monitoring is recommended.

Inotersen's safety profile is exclusively based on accounts of the NEURO-TTR study ($n = 172$) described previously and should therefore be considered preliminary until further data are available.[51] Overall, inotersen caused a notable increase in the incidence of treatment-related adverse events (78% vs. 38%) and serious adverse events (7% vs. 2%) in comparison to placebo, with glomerulonephritis and thrombocytopenia being the most severe. Specifically, 54% of patients treated with inotersen experienced a gradual decrease in platelet count (<140 000/mm^3) within the first 3 months of treatment and 3% had thrombocytopenia (<25 000/mm^3) that required discontinuation of inotersen and treatment with glucocorticoids.[51] One patient died due to intracranial haemorrhage associated with strong thrombocytopenia (<10 000/mm^3). However, weekly platelet monitoring (minimum

>50 000/mm^3) and consequential dose-reductions to 150 mg/week averted further cases of thrombocytopenia and should therefore be considered a standard procedure when using inotersen.[513945]

Another 3% of patients developed glomerulonephritis accompanied by reduced glomerular filtration rate (2%) or significant proteinuria (1%). Hence, renal function should also be monitored in intervals of 2–3 weeks.[39,45,51] Although the authors stressed that all affected patients carried V30M mutations, deducing that alternative mutations are not at risk would be a hasty conclusion.

Less severe adverse events included pyrexia, chills and mostly mild injection-site reactions (1.1%). Adverse events associated with vitamin A deficiency (explained above) were obviated by daily substitution of vitamin A (3000 IU, p.o.).[51]

Patisiran requires pre-medication with dexamethasone (i.v.), oral acetaminophen/paracetamol, an H_2-blocker (i.v.) and an H_1-blocker (i.v.) in order to reduce infusion-related reactions, which are commonly induced by pharmaceuticals in lipid nanoparticle formulations.[56,57,100] Nevertheless, mild/moderate infusion-related reactions (10–19%), resulting in back pain, abdominal pain, flushing or nausea are among the most common adverse events, the most frequent being peripheral oedema (30%).[61,62] The incidence of all other adverse events was either reduced by patisiran or similar when compared to placebo, making patisiran well tolerable overall .[61,62]

While appearing very favourable, it has to be taken into consideration that the safety profile of patisiran is based on one phase 2 and one phase 3 trial with relatively low numbers of participants ($n = 26$ and $n = 225$, respectively).[61,62] This is of particular importance, since revusiran, a subcutaneous siRNA drug developed alongside patisiran, was recently discontinued because of severe safety concerns.[101] Following promising phase 1 and 2 trials, in which revusiran was well tolerated, the ENDEAVOUR trial (phase 3) in ATTR-associated cardiomyopathy was terminated prematurely because 17 deaths occurred in the treatment arm, as opposed to two in the placebo group.[101] Analyses of adverse events showed that all-cause mortality (16.4% vs. 10.6%), heart failure (17.9% vs. 13.6%), peripheral neuropathy (5% vs. 0%) and atrial fibrillation (11.4% vs. 3%) were significantly more frequent among revusiran patients, when compared to placebo.[102] Although revusiran differed from patisiran because it employed a conjugated N-acetylglactosamine (GalNAc) ligand instead of a lipid nanoparticle formulation for hepatocyte uptake, both drugs rely on a similar mechanism of action. Hence, future trials evaluating patisiran in ATTR-associated cardiomyopathy will require thorough monitoring for similar adverse events.

Doxycycline and ***ursodeoxycholic acid*** similarly lack data for a definitive description of their safety profile. Besides one patient with gastric pain and another one with nausea and loss of appetite, a phase 2 study ($n = 20$) reported no serious adverse events.[69] However, only seven patients completed the 12-month study period, restricting the interpretability of these data. The recent retrospective study ($n = 53$) by Karlstedt and colleagues reported increased photosensitivity and/or erythema/pruritus in 8% and abdominal discomfort in 4% of patients.[67] Yet, in consideration of the nature of a retrospective study and the minor number of participants, these results can only serve as an orientation for further research into the matter.

Summary

Considering the analysis of clinical results above, the introduction of recently approved and emerging therapeutics promises the beginning of a new era in the treatment of ATTR patients. In practice, it will require careful consideration of patients' TTR status, mutation, disease stage and co-morbidities to elicit the best possible treatment for each individual.

Tafamidis plays a special role in ATTR treatment as it is the only pharmaceutical with approval for both ATTRv and -wt. While evidence for its efficacy appears to be most robust, based on currently available data, tafamidis does not seem to halt the progression of ATTR entirely. Nevertheless, it slows neurological disease progression, especially in V30M ATTRv patients and early disease stages. Patients with non-V30M mutations, advanced or late-onset disease, however, do not appear to benefit from treatment with tafamidis. In respect to cardiomyopathy, tafamidis mitigates the deterioration of functional parameters and improves overall outcome in patients with early disease stages, irrespective of TTR status. Additionally, tafamidis is well-tolerated, even for extensive treatment periods.

Patisiran appears to be the most effective treatment for ATTRv, although evidence is currently limited to the APOLLO trial. Moreover, it possesses a highly favourable safety profile. Regarding polyneuropathy, patisiran is the only pharmaceutical that showed potential to reverse deterioration of neurological function and quality of life, irrespective of mutation type and disease stage. Results on cardiomyopathy are equally promising, with patisiran reliably stabilizing structural and functional disease progression, while also reducing hospitalizations. However, when interpreting these data, it has to be taken into consideration that results of this study were based on exploratory endpoints from a relatively small cardiac subpopulation ($n = 126$). Larger trials will be needed to specifically validate the effects of patisiran on ATTR-associated cardiomyopathy.

Inotersen significantly slows but does not seem to halt neurological disease progression in ATTRv patients. Data regarding its effect on cardiomyopathy and ATTRwt are currently unavailable. Inotersen requires monitoring of renal function and platelet count at close intervals, given the risk of developing glomerulonephritis or thrombocytopenia.

The combination of doxycycline and ursodeoxycholic acid is special in the sense that both pharmaceuticals are available as generics, which may be a reason for the limited amount of studies pursued on these drugs, resulting in a lack of clinical data. However, the results discussed in this review indicate a favourable effect on disease progression and encourage further clinical evaluation.

The same is applicable to diflunisal. While evidence of a beneficial effect on polyneuropathy has been established, data on diflunisal's efficacy in cardiomyopathy are very limited and thus have to be interpreted with caution. Current data suggest that prophylaxis to prevent gastrointestinal injury and close renal monitoring are crucial to reduce the risk for side effects. Given its availability

as a generic, diflunisal therefore constitutes a relatively safe and inexpensive treatment alternative in countries where the approved treatment options discussed above may not be available.

Future considerations

Reflection on clinical applicability

Not only does the introduction of modern pharmaceuticals for the treatment of ATTR promise to address many clinical problems, but it also raises many questions.

Autopsy studies showed that 17% of patients with heart failure and preserved ejection fraction and 5% of healthy controls had wtTTR amyloid depositions.[103] Older research even suggests that 25% of patients older than 85 years had traceable wtTTR deposits in cardiac biopsies, suggesting a prevalence much higher than previously anticipated.[104] With technological advances in non-invasive diagnosis, such as magnetic resonance imaging and radionucleotide scintigraphy, a much higher proportion of those deposits will soon be detectable. However, it remains questionable whether every amyloid deposit is of pathologic significance and requires treatment. Similar to minor vascular calcifications, wtTTR amyloid deposits may be a physiologic sign of ageing to a certain extent. If that is the case, how do we determine which amount is clinically relevant and will affect outcomes? Risk stratification tools, such as reliable reference ranges for biomarkers or echocardiographic parameters, will have to be developed to identify the need for treatment.

Similar issues will become relevant for young carriers of genetic mutations that will cause ATTRv in the future. Genetic counselling is already considered standard of care, but at which point will treatment of the genetic condition need to be initiated? TTR tetramer stabilizers (i.e. tafamidis) and silencers (i.e. inotersen/patisiran) seem to bear the potential to prevent ATTRv if the condition is diagnosed early enough and the production of amyloidogenic TTR is suppressed continuously. Hence, clinical trials will need to evaluate the long-term consequences of preventive TTR stabilization/silencing.

Furthermore, realistic treatment goals for the communication with patients will need to be established. The pharmaceuticals evaluated in this review show potential to mitigate or even halt disease progression and reduce mortality. In patients with advanced disease stages however, co-morbidities may aggravate their clinical condition to the point that stabilizing disease progression will no longer be sufficient to prevent an increasing mortality. So, may disease regression be a feasible aim in the future? The pharmacological mechanism underlying TTR tetramer stabilizers and silencers (i.e. prevention of *de novo* fibril formation) does not allow for regression in the absence of a mechanism facilitating the clearance of pre-existing deposits. In fact, immunologic attempts to clear deposits through proteolytic enzymes or phagocytosis have been identified but are obviated by the compound SAP, which is present in all amyloid types, including TTR amyloid.[105] Consequently, SAP is considered crucial for the resistance of amyloid deposits to degradation and the development of anti-SAP antibodies raises hope that induction of disease regression may soon be possible.

Moreover, co-treatment with pharmaceuticals (i) inhibiting TTR production/dissociation and (ii) removing existing amyloid deposits may induce synergistic effects, which would be worthwhile exploring in clinical trials. Hence, TTR tetramer stabilizers/silencers could be combined with doxycycline and tauroursodeoxycholic acid, as the latter showed pre-clinical evidence of inducing amyloid destruction and reabsorption.[66] The same applies to EGCG,[34–36] which combined with patisiran in particular may be very promising, as all pharmacological mechanisms (i.e. TTR silencing, TTR tetramer stabilization and fibril disruption) would be employed simultaneously. Combinations of EGCG with tafamidis or inotersen are also conceivable but require careful consideration, as their efficacy may be mitigated by interactions of EGCG with human serum albumin,[38] as outlined above. Potential drug interactions may be ruled out by animal studies precipitating these clinical trials.

In contrast, co-treatment with TTR tetramer stabilizers and TTR silencers is less likely to induce synergistic effects, as the individual use of one theoretically obviates the need for the other. For example, if patisiran induces serum TTR reductions of up to 87%,[61,62] additional stabilization of the remaining 13% may be of limited value.

Design of future clinical trials

One common denominator of large clinical trials initiated and sponsored by pharmaceutical companies to gain approval for their respective drug is that they primarily focused on outcomes assessing the subjective effect on polyneuropathy (i.e. NIS-LL and Norfolk QoL-DN). As a result, with the exception of tafamidis, studies analysing the pharmaceutical effects on ATTR-associated cardiomyopathy as primary endpoints are lacking, although it is widely acknowledged as the key driver for mortality in ATTR. Hence, future clinical trials should focus on outcomes assessing cardiac function and effects on survival. Equally, more comprehensive data should be collected for ATTRwt patients, as they account for a great proportion of ATTR patients.

In more general terms, a common framework for the design of future clinical trials should be agreed on to facilitate reliable comparison of pharmaceuticals. In consideration of the biggest possible benefit to patients, we suggest that survival should be the primary outcome. Surrogate endpoints (i.e. biomarkers) should be included as secondary outcomes and used to measure treatment response in ATTR-associated cardiomyopathy. Currently NT-proBNP appears to be the most sensitive biomarker, as it significantly correlates with echocardiographic abnormalities and results of other diagnostic tools such as gadolinium-enhanced magnetic resonance imaging.[106,107] For trials on ATTR-associated polyneuropathy, validated composite scores such as the NIS-LL or the mNIS+7 seem to be reliable measures of disease severity.[93,95]

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