AMYLOID
2022, VOL. 29, NO. 3, 143–155
https://doi.org/10.1080/13506129.2022.2052838

Taylor & Francis
Taylor & Francis Group

GUIDELINE ARTICLE

Guidelines and new directions in the therapy and monitoring of ATTRv amyloidosis

Yukio Ando[a], David Adams[b], Merrill D. Benson[c,d], John L. Berk[e], Violaine Planté-Bordeneuve[f], Teresa Coelho[g], Isabel Conceição[h], Bo-Göran Ericzon[i], Laura Obici[j], Claudio Rapezzi[k], Yoshiki Sekijima[l], Mitsuharu Ueda[m], Giovanni Palladini[j] and Giampaolo Merlini[j]

[a]Department of Amyloidosis Research, Nagasaki International University, Sasebo, Japan; [b]Department of Neurology, French National Reference Centre for Familial Amyloidotic Polyneuropathy, CHU Bicêtre, Université Paris-Saclay, Le Kremlin-Bicêtre, France; [c]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA; [d]RLR Veterans Affairs Medical Center, Indianapolis, IN, USA; [e]Amyloidosis Center, Boston Medical Center, Boston University, Boston, MA, USA; [f]Department of Neurology and Amyloid Network, Hospital Henri Mondor, APHP, East-Paris University, Créteil, France; [g]Andrade's Center, Centro Hospitalar Univerisitário do Porto – Hospital de Santo António, Porto, Portugal; [h]Department of Neurosciences and Mental Health, CHULN – Hospital de Santa Maria and Faculdade de Medicina, Instituto de Fisiologia, Universidade de Lisboa, Lisbon, Portugal; [i]Division of Transplantation Surgery, Karolinska University Hospital Huddinge, Stockholm, Sweden; [j]Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy; [k]Cardiologic Center, University of Ferrara, and Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy; [l]Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan; [m]Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

ABSTRACT

The recent approval of three drugs for the treatment of amyloid transthyretin (ATTR) amyloidosis, both hereditary and wild-type, has opened a new era in the care of these diseases. ATTR amyloidosis is embedded in its pathophysiology, and the drugs target critical steps of the amyloid cascade. In addition to liver transplant, which removes the pathogenic variants, the introduction of gene silencers has allowed the suppression of both wild type and mutant transthyretin (TTR), thus extending the potential therapeutic range to wild-type cardiac amyloidosis. The kinetic stabilisation of TTR using small molecules has proved to be clinically effective both for amyloid neuropathy and cardiomyopathy. Gene silencers and kinetic stabilizers were recently approved on the basis of the outcome of phase III trials; however, comparative trials have not been performed, making it difficult to draw recommendations. Indications for liver transplantation have narrowed considerably. Here, guidelines for therapy are proposed based on expert consensus, acknowledging that the several drugs currently undergoing clinical trials will probably change in the near future the therapeutic armamentarium and, consequently, the therapeutic strategy. Indications for monitoring disease progression and drug efficacy are also provided for the management of these complexes, but now very treatable, diseases.

Abbreviations: AG10: acoramidis; AL amyloidosis: immunoglobulin light chain amyloidosis; ASO: antisense oligonucleotide; ATTR amyloidosis: amyloid transthyretin amyloidosis; ATTRv: hereditary transthyretin-related amyloid protein; ATTRwt: wild type transthyretin-related amyloid protein; COMPSAA31: composite autonomic symptom score; CSF: cerebrospinal fluid; CV: cardiovascular; FAPWTR: familial amyloidotic polyneuropathy world transplant registry; 6-MWT: six-minute walk test; mBMI: modified body mass index; mNIS: modified neuropathy impairment score; mRNA: messenger RNA; NYHA: New York Heart Association; NIS: neurologic impairment scale; Norfolk QOL-DN: Norfolk quality of life-diabetic neuropathy; NSAID: non-steroidal anti-inflammatory drug; NT proBNP: N-terminal-pro hormone brain natriuretic peptide; PND: polyneuropathy disability; QOL: quality of life; RISC: RNA-induced silencing complex; RNAi: RNA interference; R-ODS: Rash-built overall disability scale; siRNA: small interfering RNA; SC: subcutaneous; 10-MWT: 10-meter walk test; TTR: transthyretin

ARTICLE HISTORY
Received 27 October 2021
Revised 26 February 2022
Accepted 8 March 2022

KEYWORDS
ATTRv amyloidosis; monitoring; treatment; guidelines

Introduction

Transthyretin (TTR) is a 55 kDa homotetrameric protein composed of 127-residue β-sheet-rich subunits that is found mainly in the serum, cerebrospinal fluid (CSF) and aqueous fluid. The main function of TTR involves the transport of thyroxine and vitamin A-retinol binding protein complexes. Almost all serum TTR is synthesized in and secreted by the liver. The protein is also synthesized in the choroid plexus and the retinal pigment epithelium. TTR synthesized by those tissues from amyloid fibrils because of genetic mutation or conformational change of the protein.

Amyloid transthyretin (ATTR) amyloidosis is a progressive and systemic disease. The disease is divided into either hereditary (ATTRv) or sporadic (ATTRwt). Andrade first

CONTACT Giampaolo Merlini gmerlini@unipv.it Amyloidosis Research and Treatment Center, Biotechnology Research Laboratories, Department of Molecular Medicine, University of Pavia, Fondazione IRCCS Policlinico San Matteo P.le Golgi, 2825-7 Huis Ten Bosch, Pavia 19, 27100 Nagasaki, Italy; Yukio Ando andoy430@niu.ac.jp Department of Amyloidosis Research, Nagasaki International University, Sasebo 859-3298, Japan
This article has been republished with minor changes. These changes do not impact the academic content of the article.

reported a large group of patients with ATTRv (FAP ATTR Val30Met) in Portugal in 1952 [1], and other large foci have been discovered in Japan by Araki *et al.* and in Sweden by Andersson *et al.* Until 30 years ago, ATTRv amyloidosis was thought to be a disease restricted to endemic occurrence in the three countries. However, owing to progress in biochemical and molecular genetic analyses, this disease is found worldwide [2,3]. In addition to ATTRv Val30Met, the most common type, more than 150 different mutations and a deletion in TTR gene have been identified, and most of them link to ATTRv amyloidosis.

ATTRv amyloidosis was previously an intractable disease, and only symptomatic therapies were available until 1990. Liver transplantation was first developed in Sweden in 1990 to halt the progression of the disease. It has been widely accepted that life span of transplanted ATTRv patients was significantly prolonged especially when it is performed in early stage patients. However, the therapy was found to have several problems. Neuropathy and cardiomyopathy continued to progress in some patients even after liver transplantation. Moreover, ocular amyloidosis, such as glaucoma and vitreous opacity, and leptomeningeal amyloidosis cannot be prevented by liver transplantation because of the production of TTR by retinal-pigmented epithelium and choroid plexus.

In 21st century, effective disease-modifying therapies, such as stabilizers of tetrameric form of TTR, gene-silencing therapies such as siRNA and antisense therapies have been developed. There is evidence that those therapies could halt the progression of the disease, and sometimes improve the clinical manifestations of the disease. Moreover, several promising therapeutic strategies, such as antibody therapy and CRISPR-Cas9 system are now in early phase investigation as one of essential therapies for the disease.

In this guideline for ATTRv amyloidosis treatment, specialists with expertize in each therapy introduce up-to-date information which may be useful tools for daily medical care of general physician as well as amyloidologists.

Molecular mechanisms of ATTR amyloidosis and therapeutic targets

ATTR amyloid fibril formation requires rate-limiting tetramer dissociation and monomer unfolding. Amyloidogenic TTR variants destabilize the native quaternary and tertiary structures of TTR [4]. These conformational changes lead to tetramer dissociation into partially unfolded monomers, which can subsequently self-assemble into oligomers and amyloid fibrils. The energetic stability of TTR tetramer structures correlates strongly with TTR secretion efficiency and amyloidogenicity *in vitro* and thus can predict the phenotype of ATTR amyloidosis. The majority of pathogenic variants are secreted with near-wild-type efficiency and thus disease severity correlates with stability.

On the other hand, highly destabilized variants do not exhibit severe systemic amyloidosis because of efficient endoplasmic reticulum-associated degradation in the liver, but do contribute to central nervous system-dominant amyloidosis as the choroid plexus secretes them more efficiently into the CSF.

TTR proteolysis leads to the formation of highly amyloidogenic C-terminal fragments. Two distinct types of amyloid fibrils, type A and type B, have been identified in patients with ATTR amyloidosis [5]. Whereas type B fibrils are composed of full-length TTR, type A fibrils include a mixture of full-length TTR and C-terminal fragments. Type A fibrils have been observed in all TTR variants as well as in ATTRwt amyloidosis patients. In contrast, type B fibrils have only been found in patients with the Val30Met and Tyr114Cys variants. The fibril types are also correlated to phenotypic variability. In ATTRv Val30Met patients, type A fibrils are associated with late onset and myocardial involvement, while patients with type B fibrils tend to exhibit early onset and less myocardial involvement.

In ATTR amyloidosis, it is widely accepted that the amyloid fibrils themselves can cause tissue damage by direct compression, obstruction and local blood circulation failure. Carpal tunnel syndrome is a representative disorder induced by the massive deposition of amyloid fibrils and requires carpal tunnel release surgery. Vitreous opacity and glaucoma are other examples of tissue damage by amyloid fibrils, both of which are relieved by ophthalmological surgery. Cell biological studies have demonstrated low cytotoxicity for mature ATTR amyloid fibrils and increased cytotoxicity for monomeric or low molecular weight TTR oligomers [6]. Therefore, nonfibrillar TTR can also induce tissue damage in patients with ATTR amyloidosis.

Monitoring response to therapy: neurological aspects

Periodic assessment of patients treated for polyneuropathy of ATTRv is mandatory to adjust therapy, delay clinical deterioration and preserve their quality of life (QOL) [7]. In addition to peripheral neuropathy, oculo-leptomeningeal amyloidosis is sometimes accompanied, so monitoring ocular and central nervous system symptoms, such as cerebral amyloid angiopathy should be needed. The frequency of assessment needs to be adapted to the course, the severity of the polyneuropathy and response to treatment, in general every 6–12 months. Monitoring should cover all components of the polyneuropathy, including the sensory-motor and autonomic manifestations. Preferably, it should be conducted by the same physician to minimize bias of evaluation.

Sensory-motor symptoms are present in most patients. Common neuropathic symptoms to be screened are pain, paresthaesia, balance problems, walking ability and hand dexterity. The neurological examination is crucial to document the length dependent pattern of the sensory-motor deficit characteristic of polyneuropathy [8]. Additional hand involvement due to carpal tunnel syndrome is often present. All fibre functions must be tested in all four limbs. This includes temperature and pin-prick sensations which are related to unmyelinated and small myelinated nerve fibres involvement. Large myelinated nerve fibres will be tested by

assessment of muscle strength, deep tendon reflexes, vibratory sensation, light touch and position sense.

The quantitative evaluation of the sensory-motor alterations remains challenging. Combined clinical and neurophysiological scores, such as the modified neuropathy impairment score (mNIS) +7 proved useful to follow the sensory-motor course of polyneuropathy in recent clinical trials. However, these procedures are complex and time consuming, hindering their use in clinical practice. The composite clinical score NIS, combining motor function, sensory function, and tendon reflexes, is more convenient and proved reliable to monitor the polyneuropathy in several post marketing studies. The polyneuropathy disability (PND) score grades the impact of the neuropathy on ambulation. Also the six-minute walk test (6-MWT) performance and the timed 10-meter walk test (10-MWT) were pertinent end points, correlated with the polyneuropathy, longitudinally [9]. Neurophysiological tests corroborate the clinical assessment in mild to moderate polyneuropathy. Motor and sensory nerve conduction studies, including compound muscle action potentials and sensory action potentials, are performed in the four limb extremities. Investigations of the small nerve fibres, like laser evoked potentials, temperature quantitative sensory testing can be proposed but they are not broadly available.

Autonomic manifestations are, detected in ~75% of patients. They are pleomorphic involving mainly the cardiovascular, gastrointestinal and genitor-urinary systems. A systematic clinical screening is the most useful way to monitor autonomic polyneuropathy, covering its different aspects. In this setting, the compound autonomic dysfunction test integrating these different aspects, including evaluation of postural hypotension is a simple and reproducible tool to be used. Additional useful investigations include heart rate variability tests, iodine-131-meta-iodobenzylguanidine scintigraphy for cardiac dysautonomia, urodynamic test for urinary autonomic alteration and SudoScan® to detect denervated sweat glands of the palms and soles. The modified body mass index (mBMI) measuring the nutritional status may be used as an indirect evaluation of the gastrointestinal dysautonomia. However, at an advanced stage of the polyneuropathy, it may also be impacted by significant muscle wasting.

The Compass 31 questionnaire, with its 101 items proved useful to monitor longitudinally autonomic alterations in the recent trials.

On the other hand, the Kumamoto neurological scale is a compound test developed in Japanese ATTR Val30Met patients, which assesses the sensorimotor deficit, autonomic dysfunction and visceral organ impairment. It is a convenient and simple tool, which proved useful when used as a secondary endpoint in the phase III clinical trial on diflunisal.

Finally, the Rash-built overall disability scale (R-ODS) is employed to rate longitudinally the overall disability of polyneuropathy patients [10].

Overall, a common minimum set of evaluation is necessary to monitor the course of the polyneuropathy, in response to therapy. In our opinion, the monitoring should include at least once a year, an evaluation of the PND score, the 6-MWT or 10-MWT depending on the severity of the neuropathy, the composite clinical NIS score by examination, the mBMI estimation, a questionnaire on autonomic manifestation, preferably the COMPASS 31 and a functional assessment of the patient's ability in daily life, by the R-ODS. An additional array of clinical scores and investigations may be used, depending on the expertize of each neurological centre. So far, criteria for the neurological disease progression are not strictly defined. Recently, recommendations have been made on the tests aforementioned, including for each, an indicator of progression and their sensitivity to rate a progression of the neuropathy [7]. Table 1 summarizes these criteria for the common minimum set of evaluation considered in the present guidelines. Finally, a consensus on the most important end points and further refinement of the sensitivity and reliability of the different tests are needed.

Table 1. Minimum set of evaluation to monitor progression of neuropathy.[a]

Assessment	Indicator of progression	Frequency of assessment	Sensitivity to progression[b]
NIS[c]	A change of 7–16 points over 12 months or worsening of the score on 2 consecutive consultations 6 months apart Give more weight to changes in strength and less weight to changes in reflexes	6–12 months	High in Late Onset V30M
PND score	Change in disease stage Not sensitive to small changes in progression but useful to assess during monitoring visits as a change in score indicates increased functional impairment	6–12 months	Low in Early Onset V30M High in Late Onset V30M
6-MWT or 10-MWT	Change in gait speed 0.05–0.10 m/s or an increase of 30% over 12 months in the time to walk 10 m	6–12 months	High
COMPASS-31 questionnaire[c]	Increase by 1 point in a year	12 months	Low
R-ODS	Worsening of R-ODS score by 3–8 points over 12 months or worsening of the score on 2 consecutive consultations 6 months apart (questionnaire to be filled in before the consultation)	6–12 months	High

6-MWT: six-minute walk test; 10-MWT: 10-meter walk test; COMPASS-31: composite autonomic symptom score-31; mBMI: modified body mass index; NIS: neuropathy impairment score; PND: polyneuropathy disability; R-ODS: Rasch-built overall disability scale.
[a]Based on Adams D *et al.*[7].
[b]In the authors' clinical experience.
[c]These scales are non-linear so the impact of a specifc score change may differ according to the patient's starting level.

Monitoring response to therapy: cardiac aspects

Monitoring disease progression and response to therapy in cardiac amyloidosis still represents an area of uncertainty [11]. In particular, there is a lack of validated criteria to identify responders and non-responders at an early stage of treatment, in time to be able to suspend treatments and eventually switch to (or combine with) other drugs.

It is worthwhile bearing in mind some general considerations:

1. The damage produced by ATTR amyloidosis has a dual mechanism: the infiltration of organs and tissues, and a direct toxic effect of preamyloidotic oligomers on cardiac myocells.
2. The treatments that are currently available target the precursor protein and not, directly, the already formed amyloid.
3. Monitoring of therapy should, ideally, include both the direct pharmacological effect of the drug on the precursor protein and its subsequent effect on target organs, typically the heart. This is very clear and established in the treatment of light chain (AL) amyloidosis, but unfortunately much less so in ATTR amyloidosis.

Of note, the new disease-modifying drugs have opposite effects on circulating TTR (gene silencers greatly decrease it, whereas stabilizers slightly increase it).

Currently, long-term data in treated patients with ATTR cardiac amyloidosis are lacking, so clear endpoints indicating disease progression or stability are unknown. Findings from phase III trials, such as ATTR-ACT, and from subgroups with mixed phenotype in the APOLLLO and NEURO-TTR trials, may provide significant endpoints for treatment goals and identify non-response criteria. This topic has been recently focussed by a consensus panel, which provided some "expert opinion" criteria of disease progression (and their threshold values) within 6–12 months despite treatment [11]:

- Any hospitalisation for HF
- Any increase in NYHA class
- 10% decline in EQ-5D score
- 40 m decrease in 6-MWT
- 30% increase in NT proBNP with 300 pg/ml cut-off
- 30% increase in high sensitivity troponin
- LVEF decrease >5%
- >5 ml decrease in SV
- ≥2 mm increase in LV wall thickness
- New onset AF or A-V block or BB block
- Stepwise increase in diastolic dysfunction grade

Notably, these variables belong to three different categories: (i) clinical and functional endpoints, (ii) biomarkers and laboratory markers and (iii) imaging and electrocardiographic parameters. Experts recommended that one marker from each of the three domains provides the minimum requirements for assessing disease progression. More in general assessment of cardiac disease status should be part of a multiparametric evaluation in which progression, stability or improvement of other involved systems in TTR amyloidosis should also be considered. Nevertheless, monitoring response to disease-modifying therapy in cardiac amyloidosis still remains an area of uncertainty and a knowledge gap to be filled [12].

Treatments for ATTRv amyloidosis

Symptomatic therapy in ATTRv amyloidosis

Symptomatic management in ATTR amyloidosis is of major importance due to its impact on patient's QOL as well as social, economic, and psychological well-being.

Neuropathic pain

For neuropathic pain treatment, the first line therapies are serotonin-norepinephrine reuptake inhibitors as duloxetine (60–120 mg/day once a day), venlafaxine (150–225 mg/day once a day), gabapentinoids as gabapentin (300–3600 mg/day tid) and pregabalin (50–600 mg/day bid). Those should be trailed for a four- to six-week period with two weeks at the maximum tolerated dose [13]. In second-line, weak opioid analgesics as tramadol (200–400 mg/day bid) and tapentadol (50–600 mg/day) as well topical agents (i.e. lidocaine 5% plaster and capsaicin patch 8%) are recommended. As third-line drugs, strong opioids like morphine (10–120 mg/day), oxycodone (10–120 mg/day) and oral methadone (5–10 mg/day) are recommended.

Gastrointestinal disturbances

Impaired gastric emptying symptoms (early satiety, postprandial fullness, bloating, nausea, vomiting and weight loss) can be improved with dietary changes including small-volume meals with low soluble fibre and fat content. Additionally pharmacological approach with prokinetics can be used with erythromycin (50–250 mg a day, tdi) or Domperidone (10 mg bdi) if available. On acute attacks of recurrent vomiting short courses of metoclopramide (IV or IM) with prompt electrolyte and fluid supplementation can be useful [14]. Patients with obstinate constipation may benefit from osmotic laxatives and polyethylene glycol. Newer agents such as linaclotide, lubiprostone and prucalopride can be used when laxatives have failed. Diarrhoea, continuous or alternating with constipation can be treated with monthly cycles of rifaximin on days 1–7 followed by probiotics. Additionally, antidiarrhoeal opioids, i.e. loperamide, on demand can be used. Octreotide or opium tincture can be administered to patients with chronic diarrhoea refractory to loperamide. If the treatment of diarrhoea fails, the remaining option is a stoma.

Cardiac involvement

The supportive care in patients with heart failure can be done with low doses of loop diuretics (i.e. furosemide) or mineralocorticoid receptor antagonists (i.e. spironolactone

or eplerenone) in case of loop diuretics fail [15]. Beta blockers, and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, might be considered in the absence of clear contraindications, starting from low doses, with slow up-titration and close monitoring. Anticoagulation with either warfarin or the novel oral anticoagulants can be used in cases of rhythm disturbances [16]. Pacing is primarily used for significant bradycardia and certain types of atrial-ventricular blocks, as a result of myocardial tissue infiltration. Implantable cardioverter defibrillator (ICD) is not indicated as sudden cardiac death in ATTR cardiomyopathy may result from electromechanical dissociation or arrhythmias not amenable to ICD.

Orthostatic hypotension

Patients with symptomatic orthostatic hypotension may benefit with nonpharmacological interventions, as compression stockings, removal of aggravating factors as hypotensive medications (i.e. tamsulosin, carvedilol, clonidine, tizanidine, nitrates, sildenafil citrate and tricyclic antidepressants) and increasing intake of water. Pharmacological treatment involves norepinephrine replacers as midodrine (2.5–10 mg oral, tid) or droxidopa (100–600 mg oral, tid); or non-specific treatments as fludrocortisone (0.1–0.2 mg once daily), to be avoided in case of congestive heart failure, or octreotide (12.5–25 µg, up to 1–3 times/day sc).

Ocular involvement

For ocular amyloidosis management, ocular lubrification, vitrectomy or trabeculectomy are used. It is recommended to follow closely the ocular involvement in patients with relevant mutations.

Renal failure

Patients with renal involvement with normocytic normochromic anaemia, erythropoietin or IV iron can be an option. Haemodialysis is the therapeutic approach at kidney end stage disease.

Disease-modifying therapies: current and future

The main therapeutic strategies for ATTR amyloidosis are divided into three categories.

1. Inhibition of amyloidogenic TTR synthesis: liver transplantation, siRNA, antisense and CRISPR/Cas9
2. Stabilisation of the native tetramer structure of TTR: diflunisal, tafamidis and AG10
3. Removal of ATTR amyloids and/or misfolded TTR species: antibody therapy

Liver transplantation

Liver transplantation changed the concept of treatment for ATTRv amyloidosis from being a disorder that most patients, and sometimes also their doctors, preferred to have confirmed only after symptoms developed because no curable treatment was available, to one that involved an interest in early diagnosis and a multi-disciplinary approach. Liver transplantation as a treatment modality presently represents the longest follow-up, more than 20 years, for ATTRv patients with polyneuropathy.

In Stockholm, where the first liver transplantation for ATTRv amyloidosis was performed in 1990, the Familial Amyloidotic Polyneuropathy World Transplant Registry (FAPWTR) was initiated to accumulate outcome data for the procedure and obtain possible predictive factors for when to recommend liver transplantation and when the treatment should not be recommended [17]. The early information was mainly related to the Val30Met-variant and indicated that liver transplantation seemed to halt the progress in the majority of the patients, if not in all. Later, it was seen that late onset, i.e. after the age of 50, of ATTRv and especially late onset male Val30Met patients had a less favourable prognosis after liver transplantation. Quite early, it was evident that the different mutations could affect the post-transplant prognosis. Therefore, outcome data were analysed separately for patients with Val30Met and other variants, the non-Val30Met. In general, the Val30Met patients had superior survival when compared to non-Val30Met patients. It was later seen that some mutations had similar good outcome as Val30Met but that mutations existed where recommendation for transplantation could not be done such as oculoleptomeningeal variants since liver transplantation only replaces most of the extra-cerebral production of the variant. Several early mistakes for this rare indication were noted since at most transplant centres, outside the endemic areas, these patients were extremely rare and needed considerations other than what was typical for standard liver transplantation. One such learning was that these patients tended to be downgraded on the waiting list because the transplant was usually not considered urgent enough to compete with end-stage liver cirrhosis thus transplanting in a more advanced stage of the disease. After having evaluated waiting time for transplantation and the mBMI in the FAPWTR as indication on disease progress it was evident that these patients needed special handling to avoid delaying liver transplantation and improve post-transplant outcome. A mBMI of more than 700 and a duration of disease before liver transplantation <7 years were early positive prognostic factors. Liver transplantation due to an abnormal replacement of one amino acid for another in one of all the proteins produced by the liver has to be regarded as exceptional. However, at that time these patients could only calculate the time to death and hope for a slow progress with no available treatment. Many patients had seen their older relatives slowly disappear from decent life. From being a diagnosis wanted to be received as late as possible it became a diagnosis that should be discovered early to optimize treatment efficiency. These needs for early diagnosis and awareness among professionals handling the patients has maintained into the pharmacotherapy era.

Although thousands of patients have undergone liver transplantation and the life expectancy has improved significantly among them, maybe the most important effect of

liver transplantation on this disorder is the international awareness of the disease today with a focus on early diagnosis and novel research to find more specific and less risky treatment options. With the introduction of potentially effective pharmacotherapeutic drugs the number of liver transplantations has steadily declined over a 10-year period to make, as previously postulated, the 2020s decade to the post-transplant era.

Diflunisal

Diflunisal, an FDA approved generic non-steroidal anti-inflammatory drug (NSAID), is one of two thyroxine-mimetic TTR tetramer stabilizers proven effective for hereditary TTR amyloid polyneuropathy by randomized controlled trial. First marketed (1981) as treatment for osteoarthritis, investigators at The Scripps Research Institute demonstrated that diflunisal stabilizes TTR by occupying the thyroxine binding sites at the dimer–dimer interface of circulating TTR tetramers, effectively preventing release and misfolding of TTR monomers to amyloid fibrils.

An international, placebo-controlled randomized clinical trial enrolled 130 patients with hereditary TTR amyloid polyneuropathy with a spectrum of nerve injury (>30% requiring canes, crutches or wheelchair) and TTR gene mutations (45% non-Val30Met ATTR), employing a quantitative neurologic scale (NIS + 7) previously validated in diabetic polyneuropathy [18]. Diflunisal inhibited progression of neuropathy over 24 months by 69% while improving mental and physical QOL. Fully 29% of diflunisal treated patients exhibited complete neurologic stability by NIS + 7 measures over two years. Diflunisal proved highly effective across gender, TTR mutations, and severity of neurologic disease. In patients meeting study entry criteria (creatinine clearance > 30 ml/min/1.73 m^2, < class IV New York Heart Association (NYHA, heart failure, and no anticoagulants), diflunisal 250 mg BID was well tolerated with or without proton pump inhibitor/H1 blocker. Diflunisal-related adverse events resulted in study drug discontinuation in only 4/64 (6%) of treated patients (heart failure, gastrointestinal bleeding, nausea and glaucoma) versus 2/66 (3%) of the placebo group (headache and renal failure) with surveillance renal chemistries performed after 1, 3, 6, 12 and 24 months of treatment. Renal and gastrointestinal adverse events were statistically indistinguishable between treatment groups.

Subsequent single-centre reports document the safety of diflunisal treatment in patients with ATTR amyloid cardiomyopathy when used in a select population (eGFR >40 ml/min/1.73 m^2 without excessive fluid overload). Collectively, 165 ATTR cardiomyopathy patients were treated with diflunisal for a mean of 11–38 months with 7.5%−13% incidence of treatment discontinuations due to drug-related adverse events. Over the period of observation in each of these retrospective reports, echo parameters (IVSd, LVEF and GLS) of infiltrative disease, cardiac biomarkers and survival improved in patients receiving diflunisal versus those receiving no treatment.

In summary, diflunisal remains an underutilized TTR tetramer stabiliser with disease modifying capacities for patients with familial ATTR amyloidosis complicated by polyneuropathy or cardiomyopathy. The benefit of TTR protein stabilizers in patients receiving TTR gene silencers remains unproven.

Tafamidis

Tafamidis is a small molecule specifically developed to stabilize TTR as it binds competitively to one of the thyroxine binding sites of the tetramer. The clinical development of Tafamidis included a double-blind RCT comparing 20 mg oral capsule against placebo (1:1), for 18 months, in patients with early disease and the Val30Met mutation.

The Tafamidis trial did not achieve statistical significance in the two primary outcome measures, NIS-LL categorical analysis of change from baseline in the ITT population and Norfolk score change from baseline in the same population. However, in the EE population, significantly more tafamidis patients than placebo patients were NIS-LL responders (60.0% vs. 38.1%; $p = .041$), and tafamidis patients had better-preserved TQOL (0.1 vs. 8.9; $p = .045$). Significant differences in several secondary endpoints also favoured tafamidis. TTR was stabilized in 98% of tafamidis and 0% of placebo patients ($p = .0001$). Adverse events were similar between groups [19]. These results supported tafamidis approval in Europe, in 2011, and later in several Asian and South American countries. The indication is for daily use of a 20 mg capsule, to stop or decrease neuropathy progression in stage 1 symptomatic patients (fully ambulatory) with hereditary ATTR amyloidosis with any mutation [19].

Besides the pivotal double-blind clinical trial, one smaller open-label study included 21 patients with eight different mutations and followed them for more than one year. Results were difficult to interpret because there was no control arm: TTR stabilisation, the primary outcome measure, was demonstrated across all mutations, but all patients showed disease progression, unclassifiable due to the lack of a control group [20].

The evaluation of long term Tafamidis treatment (up to 6 years) in Portuguese stage 1 patients with the Val30Met mutation, showed that patients with early disease (NIS ≤ 10) and female patients had the best response to treatment with prolonged and complete stabilisation of the disease. Some patients with a poorer response showed a low concentration of Tafamidis in blood after one year of treatment raising the question of the need to adjust the treatment dose [21].

Across all the studies with Tafamidis 20 mg no major safety issues were raised.

A clinical trial with Tafamidis in two different doses, 20 and 80 mg, was implemented across the world, for patients with TTR related cardiomyopathy, either hereditary or wild-type, with 30 months duration. The primary outcome measures, a hierarchical combination of all-causes mortality and frequency of cardiovascular related hospitalisations, showed significant differences between the 264 patients who received tafamidis and the 177 patients who received placebo

($p < .001$). Tafamidis was associated with lower all-cause mortality than placebo (78 of 264 [29.5%] vs. 76 of 177 [42.9%]) and a lower rate of cardiovascular-related hospitalisations, with a relative risk ratio of 0.68 (0.48 per year vs. 0.70 per year). At month 30, tafamidis was also associated with a lower rate of decline in distance for the 6-MWT ($p < .001$) and a lower rate of decline in KCCQ-OS score ($p < .001$). When comparing patients in different NYHA classes those in NYHA III had lower mortality than placebo patients but showed increased hospitalisation risk. A possible explanation for this result may be that due to increased survival these severe patients need more frequent hospital support. When comparing the two doses (20 mg vs. 80 mg) efficacy the higher dose showed more robust effect on mortality. No safety issues were detected with either dose [22].

Tafamidis 61 mg (a different formulation of free acid form of tafamidis equivalent to 80 mg of previous formulation of meglumine salt of tafamidis) was approved worldwide to treat ATTR amyloidosis with cardiomyopathy, either inherited or wild-type.

Tafamidis was show to be present in the CSF and in the vitreous body of patients submitted to vitrectomy. The concentration in the eye is negligible, but the concentration in the CSF could be able to stabilize the smaller amount of TTR present. Clinical impact should not be construed at present. Further laboratory studies and clinical evaluation are needed to know if tafamidis could be useful to prevent or treat CNS involvement [23].

In conclusion Tafamidis is a safe drug easy to manage, for patients and doctors. It is the only approved treatment for ATTR amyloidosis with cardiomyopathy, either inherited or wild-type and is a useful drug for the treatment of patients with polyneuropathy in very early stage especially early onset Val30Met variant.

RNAi therapeutics

Progression of amyloid polyneuropathy and cardiomyopathy after liver transplantation in hereditary TTR amyloidosis due to ATTRwt deposition in existing amyloid deposits encouraged development of treatment able to block liver production of both mutated and wild-type TTR. RNA interference (RNAi) patisiran uses an endogenous cellular mechanism for controlling gene expression in which small interfering RNAs (siRNAs) that are bound to the RNA-induced silencing complex (RISC) mediate the cleavage of target messenger RNA (mRNA) of both ATTRv and ATTRwt. The ability to turn siRNAs into drugs required successive chemical processes and facilitate effective delivery to liver by formulation of lipid nanoparticles. That resulted in a robust and durable reduction in genetic expression of hepatocyte.

In the phase III trial APOLLO A, 225 patients from 19 countries, with ATTRv polyneuropathy with 39 TTR variants were randomly assigned in a 2:1 ratio, to receive intravenous patisiran (0.3 mg/kg) or placebo once every three weeks. The primary end point was the change from baseline in the modified Neuropathy Impairment Score + 7 (mNIS + 7) at 18 months. Other assessments included the Norfolk QOL–Diabetic Neuropathy (Norfolk QOL-DN) questionnaire, 10-MWT, COMPASS31 autonomic score and modified body-mass index. The mean mNIS + 7 at baseline was 80.9 in the patisiran group and 74.6 in the placebo group; the least-squares mean change from baseline was −6.0 versus 28.0 ($p < .001$) at 18 months. The mean baseline Norfolk QOL-DN score was 59.6 in the patisiran group and 55.5 in the placebo group; the least-squares mean change from baseline was −6.7 versus 14.4 ($p < .001$) at 18 months. Improvement from baseline of mNIS + 7 concerned 56% in patisiran versus with placebo. The treatment effect was significant for all subgroups regardless of NIS, variant Val30Met or not, age and stage of disease. Clinically, pertinent endpoints walking 10-MWT, COMPASS31 and Norfolk QOL improved from baseline and disability score RODS remained stable. In the patisiran group, the reduction in serum TTR levels was rapid and sustained 81% over a period of 18 months. Common adverse events were mild or moderate in severity and those more frequently with patisiran than with placebo included peripheral edoema and infusion-related reactions. No clinically relevant changes in laboratory values related to patisiran were observed during the trial. There was no death related to patisiran [24]

A multicentre, open-label extension (OLE) trial enrolled patients who had completed the phase III APOLLO study and tolerated the study drug. Eligible patients received patisiran 0·3 mg/kg by IV infusion every three weeks. At 12 months, mean mNIS + 7 score improved from global OLE enrolment in the APOLLO-placebo group and were sustained from parent study baseline with treatment in the global OLE (APOLLO-patisiran). The most common treatment-related adverse event was infusion-related reactions. The frequency of deaths in the global OLE was higher in the APOLLO-placebo group (27%) who had a higher disease burden than the APOLLO-patisiran (7%) groups. A sustained reduction in mean serum TTR concentration was observed in the APOLLO-placebo group upon patisiran treatment with a mean percentage TTR reduction of 78.7% at month 6 and was maintained in APOLLO-patisiran [25].

In an exploratory analysis of APOLLO A, in a prespecified cardiac subpopulation patients with a baseline LV wall thickness of 13 mm or more, patisiran reduced mean left ventricular (LV) wall thickness, interventricular septal wall thickness at month 18 compared with placebo. Patisiran improved LV global longitudinal strain (LVGLS) N-terminal prohormone of brain natriuretic peptide compared with placebo [26], and decreased a composite end point of cardiac hospitalisations and all-cause mortality which was assessed in a *post hoc* analysis. Apollo B (ClinicalTrials.gov Identifier: NCT03997383) is an ongoing phase III, Randomized, Double-blind, Placebo-controlled Multicentre Study to Evaluate the Efficacy and Safety of Patisiran in Patients With ATTR Amyloidosis With Cardiomyopathy with Primary Outcome Measures: Change from Baseline at Month 12 in 6-MWT, as Secondary Outcome Measures: Change from Baseline at Month 12 in Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-

OS) Score and Composite Endpoint of All-Cause Mortality and Frequency of All-Cause Hospitalisations and Urgent HF Visits. Medicine under development

Vutrisiran (previously ALN-TTRSC02) is a second-generation investigational RNAi therapeutic under development for the treatment of ATTR amyloidosis. Like patisiran, it contains an siRNA that targets a sequence within the TTR mRNA which is conserved across wt and all known TTR variants. However, the vutrisiran siRNA utilizes enhanced stabilisation chemistry and is conjugated to a triantennary GalNAc ligand, with the aim of enabling infrequent, subcutaneous dosing. In a phase I, randomized, single-blind, placebo-controlled, single ascending dose study evaluated the pharmacodynamics, pharmacokinetics and safety profile of subcutaneously administered vutrisiran (5–300 mg) in healthy subjects ($n = 80$). Vutrisiran treatment achieved potent and sustained TTR reduction in a dose-dependent manner, with mean maximum TTR reduction of 57–97%, maintained for ≥90 days post dose [27].The purpose of Helios-A study is to evaluate the efficacy and safety of vutrisiran in patients with ATTRv amyloidosis. Participants will receive vutrisiran or the reference comparator patisiran during the Treatment Period. This study will use the placebo arm of the APOLLO study (NCT01960348) as an external comparator for the primary and most other efficacy endpoints. Primary endpoint: Change from Baseline in the Modified Neurologic Impairment Score +7 (mNIS + 7) at Month 9.

Antisense

ATTR therapy with antisense oligonucleotide (ASO) technology to decrease hepatic TTR amyloid precursor protein synthesis was conceived in 2000. The basis of this approach to inhibiting hepatic synthesis of a particular protein involves destruction of mRNA for that protein with short nucleotide oligomers designed to bind to mRNA by a Watson-Crick hybridisation mechanism of action. For TTR, ASO compounds which bind to the 3′ untranslated region of TTR effectively activate RNase H1 to degrade TTR mRNA and decrease translation. Development of a therapeutic ASO specific for human TTR started with development of the human ATTR Ile84Ser transgenic mouse model, which allowed demonstration that TTR specific ASO could reduce human hepatic TTR production. This led to safety studies in human subjects and subsequent placebo-controlled studies in ATTRv patients, which demonstrated highly significant results with primary goals of NIS and QOL measurements [28]. This study led to the approval of inotersen for the therapy of ATTRv amyloidosis in October 2018. A total of 172 patients (112 in the inotersen group and 60 in the placebo group) received at least one dose of a trial regimen, and 139 (81%) completed the intervention period. Both primary efficacy assessments favoured inotersen: the difference in the least-squares mean change from baseline to week 66 between the two groups (inotersen minus placebo) was −19.7 points (95% confidence interval [CI], −26.4 to −13.0; $p < .001$) for the mNIS + 7 and −11.7 points (95% CI, −18.3 to −5.1; $p < .001$) for the Norfolk QOL-DN score. These improvements were independent of disease stage, mutation type, or the presence of cardiomyopathy. There were five deaths in the inotersen group and none in the placebo group. The most frequent serious adverse events in the inotersen group were glomerulonephritis (in 3 patients [3%]) and thrombocytopenia (in 3 patients [3%]), with one death associated with one of the cases of grade 4 thrombocytopenia. Thereafter, all patients received enhanced monitoring. Now treatment of patients with TTR cardiomyopathy, hereditary or "wild-type," is underway with a modified inotersen ASO (ION682884), which poses to be more effective with lower dosage and interval of administration.

Medicines under development

Acoramidis

Among investigational treatments, acoramidis (previously known as AG10) is a highly selective, orally bioavailable, small-molecule TTR stabilizer specifically designed to optimize the occupancy of thyroxine binding sites and enhance tetramer kinetic stability. Similarly to the naturally occurring, non-amyloidogenic Thr119Met mutation, acoramidis strengthens the dimer–dimer interaction, preventing tetramer dissociation, misfolding and aggregation. Preclinical data in plasma have shown that acoramidis binds to TTR with higher selectivity than thyroxine and other monovalent TTR stabilizers.

The safety, tolerability, pharmacokinetics and pharmacodynamics of acoramidis were recently evaluated in a phase II randomized, placebo-controlled trial in patients with ATTR cardiomyopathy and symptomatic heart failure. Patients were randomized to receive AG10 400 mg, AG10 800 mg or placebo twice daily for 28 days. The drug was well tolerated with no safety signals at both doses, consistent with the previous phase I study in healthy volunteers. Patients treated with acoramidis achieved target concentrations in plasma resulting in near-complete stabilisation of TTR compared to placebo. At the higher dosage, the average steady-state concentration showed less intersubject variability and was associated with more than 90% TTR stabilisation, as assessed by *ex vivo* assays [29]. This effect did not differ between mutant and wild-type TTR although the sample size was small. Moreover, serum TTR concentration significantly increased from baseline in both acoramidis treatment groups compared to placebo, being interpreted as indirect evidence of TTR stabilisation. Consistently, a dose-dependent increase in TTR concentration was observed in patients treated with tafamidis [30]. Currently, two phase III studies are ongoing. The ATTRibute-CM trial is a double blind, placebo-controlled study that evaluates the safety and efficacy of acoramidis in patients with hereditary or wild-type ATTR cardiomyopathy and heart failure. Patients are randomized in a 2:1 ratio to receive acoramidis 800 mg BID or placebo for 30 months. Key primary endpoints are the change from baseline to Month 12 in distance walked during the 6-MWT and a hierarchical combination of all-cause

mortality and CV-related hospitalisation over the 30-month treatment period. Enrolment was completed in September 2020. The 12-month analysis of the ATTRibute-CM trial reported that the primary endpoint of the 6-MWD was not met. However, improvements were observed in the Kansas City Cardiomyopathy Questionnaire Overall Score (KCCQ-OS, nominal $p < .05$), in NT-proBNP (median +0.6% vs. +24.3%, nominal $p < .05$), and in serum TTR concentration (mean +38.5% vs. −0.7%, nominal $p < 0.01$), a measure of TTR stabilisation. Acoramidis was generally well-tolerated [31].

Tolcapone

Another kinetic stabilizer is tolcapone, a drug approved for the treatment of Parkinson's disease, which has a very good stabilising effect and capacity to cross the blood–brain barrier, thus offering hope for the treatment of leptomeningeal manifestations of ATTRv amyloidosis [32]. In a phase IIa study, tolcapone induced a robust stabilisation of plasmatic TTR in all patients studied and the drug was well tolerated [33].

CRISPR/Cas9

Using the CRISPR/Cas9 technology (NTLA-2001), the TTR gene can be knocked-out in patients with ATTR amyloidosis with a single administration. A phase I study performed on a small group of patients with ATTRv amyloidosis and polyneuropathy, administration of NTLA-2001 was associated with decreased serum TTR protein concentration and was well tolerated (Figure 1) [34].

Antibody therapies

To disrupt and remove tissue ATTR amyloid deposits, therapeutic amyloid-directed antibodies are now under investigations. To date, potential therapeutic monoclonal antibodies (mAbs) targeting two epitopes, comprising residues 89–97 (β-strand F) and 115-124 (β-strand H), were developed. Those regions are more exposed in the TTR monomer than in the dimer or tetramer and reportedly contribute TTR amyloid formation *in vitro*. The mAbs against those two cryptic epitopes, TTR89-97 and TTR115-124, specifically reacted with ATTR amyloid deposits *in vitro* and in human tissue, but those mAbs did not react with native TTR tetramers *in vitro* and in human serum. Those mAbs also inhibited ATTR amyloid formation *in vitro*. T24, targeting TTR115-124, inhibited TTR deposits in ATTRv amyloidosis model rats having human ATTR Val30Met [35].

In phase I clinical trial, PRX004, targeting TTR89-97, was found to be well-tolerated and generally safe at all dose levels. In the phase I, open-label, multicentre dose-escalation study enrolled 21 patients with ATTRv amyloidosis to receive PRX004 intravenously once every 28 days for up to three infusions in the dose escalation phase of the study. Patients were enrolled into 1 of the following 6 PRX004 dose cohorts: 0.1, 0.3, 1, 3, 10 and 30 mg/kg, starting with the lowest dose. At month 9, all the 7 evaluable patients showed improvement/slower progression in neuropathy versus disease natural history and improvement in GLS, suggesting that amyloid targeting with PRX004 provides therapeutic benefit [36].

More recently, selective anti-ATTR therapeutic antibody NI301A, a recombinant human monoclonal immunoglobulin G1, was cloned from memory B cell repertoires derived from healthy elderly subjects [37].

Summary and perspectives

The therapy of ATTR amyloidosis is embedded in its pathophysiology. The three therapeutic pillars are: TTR knockout, TTR stabilisation, and amyloid removal (Table 2). The first two pillars comprise four disease modifying therapies that are in clinical practice, liver transplantation, tafamidis,

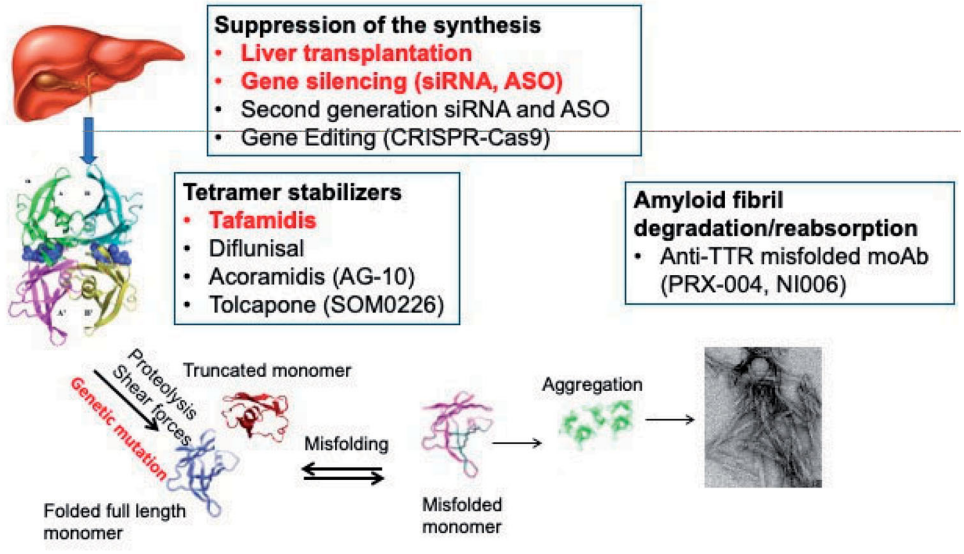

Figure 1. The amyloid cascade offers several key targetable steps. The figure reports drugs available in the clinic in red and those in clinical trials in black. siRNA: short interfering RNA; ASO: antisense oligonucleotides; CRISPR: clustered regularly interspaced short palindromic repeats; Cas9: CRISPR associated protein 9.

Table 2. Medicines for the treatment of ATTR amyloidosis.

Drug	Phase of Study	Side effects	Approval
Stabilizers			
Diflunisal	Phase II/III	• Renal dysfunction • Bleeding • Hypertension • Fluid retention	Commercial drug without specific approval for ATTR amyloidosis
Tafamidis meglumine (Vyndaqel)	Phase II/III in ATTRv-PN (NCT00409175)	No known side-effects or interactions	• Vyndaqel approved for ATTRv-PN in stage I patients by EMA and in other countries (see text)
Tafamidis free acid (Vyndamax)	Phase III in ATTRwt and ATTRv-CM (ATTR-ACT, NCT01994889)		• Vyndaqel and Vyndamax approved for ATTR-CM by FDA and EMA and in other countries
Acoramidis/AG10	Phase III in ATTRwt and ATTRv (ATTRIBUTE-CM; NCT03860935)	No known side-effects or interactions	n/a
Knockdown/silencing *TTR*			
Inotersen (Tegsedi)	Phase II (NCT03702829); not being pursued for ATTR-CA	• Thrombocytopenia • Glomerulonephritis • Vitamin A deficiency	Approved for ATTRv-PN by FDA and EMA and in other countries
Patisiran (Onpattro)	Phase III (APOLLO-B NCT03997383)	• Infusion reactions • Vitamin A deficiency	Approved for ATTRv-PN by FDA and EMA and in other countries
Vutrisiran	Phase III (HELIOS-B; NCT04153149	• Vitamin A deficiency	n/a
AKCEA-TTR-LRx/ ION 682884	Phase III (Cardio-TTRansform; NCT04136171)	• Vitamin A deficiency	n/a
NTLA-2001 (CRISPR-Cas9–based gene-editing therapy)	Phase I in ATTRv-PN (NCT04601051)	• Infusion-related reaction	n/a
Antiamyloid mAbs			
PRX-004	Phase I (NCT03336580)	• Fall • Anaemia • Upper respiratory tract infection • Back pain • Constipation • Diarrhoea • Insomnia	n/a
NI006	Phase I (NCT04360434)	n/a	n/a

inotersen and patisiran for ATTRv polyneuropathy and only one, tafamidis, for ATTR cardiomyopathy. Clinical trials are ongoing to assess the impact of TTR gene silencers on ATTR cardiomyopathy and of TTR gene editing in ATTRv polyneuropathy. The introduction of potentially effective pharmacotherapeutic drugs, which are most active in patients who are the best candidates for liver transplantation, i.e. young, early stage patients with Met30, has dramatically reduced the role of liver transplantation in the care of ATTR amyloidosis. Although great advances have been achieved for the first two pillars, with several new drugs in clinical trials, effective amyloid removal is still unaccomplished and further research for new workable amyloid fibril targets is needed.

Eradication of the circulating precursor has proven extremely effective first in AL amyloidosis and later in ATTR amyloidosis. Further drug modifications of siRNA and ASO for a more specific hepatic uptake, and higher and longer efficacy are the next steps under clinical investigation. Remarkably, the possibility to knockout the TTR gene using the CRISPR-Cas9 technology, as recently reported, gives hope to cure this disease [34]. Long-term effects of permanent knockdown of TTR are unknown and must be monitored but when we are facing a patient with a severe and threatening disease like this; efficacious treatment must be the priority.

Oculo-meningovascular amyloid deposition is still an unmet need and drugs capable to cross the blood-retina and blood-brain barriers are needed. Tolcapone may represent an option for leptomeningeal amyloidosis.

These new disease modifying drugs are extremely expensive, and, although the price varies among countries, the present annual cost in the US for Tafamidis is $225,000 and approximately $450,000 for gene silencing drugs (Inotersen and Patisiran) [38]. Combination therapy of drugs with different mechanisms of action could also be beneficial but again, controlled studies are necessary to confirm this theoretical hypothesis. However, the high costs of these drugs raise concern on drug accessibility that is of critical importance, particularly for low-income countries [39].

Despite the recent giant achievements in the understanding of the molecular mechanisms of ATTR amyloidosis, and in the development of new effective medicines, with the approval of three drugs in the last two years, new challenges need to be overcome to further improve the care of this disease. To evaluate each medicine benefits, accurate biomarkers of progression and treatment response are critical. Several markers and scores have been proposed; however, they have limitations such as subjectivity or complexity in assessment, and further research is needed in this critical area. Patient engagement studies that include relevant patient-centric endpoints, such as patient-reported outcomes are also needed [40]. The definition of functional markers of outcome requires a choral effort involving patient associations, clinical investigators, and scientific societies with the contribution of pharma companies.

The lack of adequate head-to-head clinical trials comparing the three drugs presently approved makes it difficult to select the appropriate compound for a specific patient. Furthermore, real-life long-term data are required to define individual predictors for treatment response.

The increasing availability of treatment options permits now to fully prevent amyloid end organ damage, and all available treatments are best effective in the early stage of ATTR amyloidosis. Therefore, early diagnosis is vital, however, late diagnosis is still very common [41]. For sporadic cases, early diagnosis is still an unmet need, despite awareness regarding the disease has markedly improved in recent years and machine learning offers hope for enhancing our diagnostic capability [42]. How to monitor mutation carriers and how to define the best time to start therapy is still a matter of investigation due to the lack of solid data. The future of ATTR amyloidosis appears bright with three drugs already approved and several others in the pipeline. However, while available treatments have shown to be able to halt or slow down the disease progression, only a small proportion of patient recovers the function of the target organs. Furthermore, it is not clear if the reduction of circulating TTR concentration up to 80–90%, achievable with current gene silencing, is sufficient to arrest the amyloid deposition, particularly in TTR variants with high amyloidogenicity potential, considering the seeding effect of preformed amyloid fibrils [43]. Deeper understanding of the molecular mechanisms involved in fibril formation and organ damage is necessary for curing the disease.

Limitations

These guidelines represent opinions of the key and experienced leaders in the field of ATTRv amyloidosis. Since more than 150 different points of mutations in TTR gene have been identified and special phenotypes of ATTRv are present, the guidelines cannot refer to all of them.

Disclaimer

These guidelines are meant to assist clinicians in making decisions regarding treatment of patients with ATTRv amyloidosis. Adherence to ATTRv treatment recommendations will not ensure successful treatment in every situation. The ultimate judgement regarding the propriety of any specific therapy must be made by the physician and the patient in light of all the circumstances presented by the individual patient, and the known variability and biological behaviour of the disease. These guidelines reflect the best available data at the time this document was prepared. The results of future studies may require revisions to the recommendations in this document to reflect new data.

Disclosure statement

Ando Y: Consultancy for Alnylam Pharmaceuticals, Prothena Co. and Pfizer Inc.

Adams D: Consultancy for Alnylam Pharmaceutical, Bridgebio and Pfizer Inc.

Benson MD: Consultancy for Ionis Pharmaceuticals

Berk JL: Scientific advisory board: Corino Therapeutics; Ad hoc advisory committee: Eidos/BridgeBio, Ionis Pharmaceuticals; Consultancy: Alnylam Pharmaceutical

Planté-Bordeneuve V: None

Coelho T: Past and current investigator in clinical trials sponsored by FoldRx, Pfizer, Alnylam, Ionis, Prothena and Eidos–Institution was paid per protocol. Consultant for Pfizer, Alnylam, Ionis, Akcea, Sobi, Eidos and Prothena pro bono. Has spoken on behalf of Pfizer, Alnylam, Glaxo and Prothena at Scientific Meetings and received financial compensation. Pfizer, Alnylam, Ionis and Biogen supported expenses with travel, accommodation and registrations for scientific meetings.

Box 1. Recommendations for the treatment of ATTRv amyloidosis

RECOMMENDATIONS

Ideally, ATTR amyloidosis should be treated as soon as disease onset is diagnosed to achieve the best therapeutic response and to prevent significant end organ damage

Lack of head-to-head comparison of effective therapies makes it difficult to select best therapy

- Patients with pure neuropathy should be treated either with tafamidis (in early stage 1) or with patisiran or inotersen gene silencers (in stage 1 and 2), according to physician and local health authority criteria.
  *Level of evidence II, Grade for recommendation A**
- Patients with mixed phenotype, neuropathy and cardiomyopathy (defined by evidence of cardiac involvement by echocardiography with an end-diastolic interventricular septal wall thickness > 12 mm), can be treated with tafamidis because it is the only drug approved for cardiomyopathy.
  Level of evidence II, Grade for recommendation A
  However, in patients with mixed phenotype, physicians may prescribe a gene silencer based on rationale and indirect, although not formally confirmed, evidence of benefit.
- Off-label use of diflunisal may be considered if other options are not available.
  Level of evidence II, Grade for recommendation A
- Patients with isolated cardiomyopathy should be treated with tafamidis
  Level of evidence II, Grade for recommendation A
- Multidisciplinary supportive therapy is essential
  Level of evidence IV, Grade for recommendation D

The combination of TTR stabilizers and gene silencers is expected to be synergistic. However, there are no trials supporting this combination, and also considering the high cost of most of these drugs, it is recommended to explore the efficacy of the combination within controlled clinical trial.

Several phase III trials are near conclusion and new drug approvals with new indications are expected soon. This will change the therapeutic landscape and recommendations

*The criteria for the "level of evidence" and "grade for recommendation" are reported in Appendix A.

Conceição I: IC reports primary investigator and consultancy for Alnylam Pharmaceuticals, Pfizer Inc. and IONIS Pharmaceuticals

Ericzon BG: Advisory board member of Pfizer Inc. and Alnylam Pharamaceuticals

Obici L: Reports speaker honoraria from Pfizer Inc, SOBI, Akcea and Alnylam Pharmaceuticals.

Rapezzi C: Institutional Research Grants from Pfizer Inc.; Board and Congress honoraria from Pfizer Inc., Alnylam Pharmaceuticals, Sanofi, Novartis Pharmaceuticals.

Palladini G: Janssen (Advisory board, honoraria), Protego (Advisory board, honoraria), Zentalis (Advisory board, honoraria), Pfizer (Honoraria), Sebia (Honoraria), Siemens (Honoraria), The Binding Site (Honoraria)

Sekijima Y: Honoraria and research funding from Alnylam Pharmaceuticals and Pfizer Inc.

Ueda M: Honoraria and research funding from Alnylam Pharmaceuticals, Prothena Co. and Pfizer Inc.

Merlini G: Advisory board member for Attralus, Janssen Pharmaceuticals, Novo Nordisk and Pfizer Inc.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

John L. Berk http://orcid.org/0000-0002-1768-2373
Isabel Conceição http://orcid.org/0000-0003-0934-9631

References

[1] Andrade C. A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain. 1952;75(3):408–427.
[2] Hawkins PN, Ando Y, Dispenzeri A, et al. Evolving landscape in the management of transthyretin amyloidosis. Ann Med. 2015;47(8):625–638.
[3] Adams D, Koike H, Slama M, et al. Hereditary transthyretin amyloidosis: a model of medical progress for a fatal disease. Nat Rev Neurol. 2019;15(7):387–404.
[4] Sekijima Y, Wiseman RL, Matteson J, et al. The biological and chemical basis for tissue-selective amyloid disease. Cell. 2005; 121(1):73–85.
[5] Ihse E, Rapezzi C, Merlini G, et al. Amyloid fibrils containing fragmented ATTR may be the standard fibril composition in ATTR amyloidosis. Amyloid. 2013;20(3):142–150.
[6] Reixach N, Deechongkit S, Jiang X, et al. Tissue damage in the amyloidoses: Transthyretin monomers and nonnative oligomers are the major cytotoxic species in tissue culture. Proc Natl Acad Sci U S A. 2004;101(9):2817–2822.
[7] Adams D, Algalarrondo V, Polydefkis M, et al. Expert opinion on monitoring symptomatic hereditary transthyretin-mediated amyloidosis and assessment of disease progression. Orphanet J Rare Dis. 2021;16(1):411.
[8] Plante-Bordeneuve V, Said G. Familial amyloid polyneuropathy. Lancet Neurol. 2011;10(12):1086–1097.
[9] Vita GL, Stancanelli C, Gentile L, et al. 6MWT performance correlates with peripheral neuropathy but not with cardiac involvement in patients with hereditary transthyretin amyloidosis (hATTR). Neuromuscul Disord. 2019;29(3):213–220.
[10] Pruppers MH, Merkies IS, Faber CG, et al. The Val30Met familial amyloid polyneuropathy specific rasch-built overall disability scale (FAP-RODS(©)) J Peripher Nerv Syst. 2015;20(3): 319–327.
[11] Garcia-Pavia P, Bengel F, Brito D, et al. Expert consensus on the monitoring of transthyretin amyloid cardiomyopathy. Eur J Heart Fail. 2021;23(6):895–905.
[12] Griffin JM, Rosenblum H, Maurer MS. Pathophysiology and therapeutic approaches to cardiac amyloidosis. Circ Res. 2021; 128(10):1554–1575.
[13] Szok D, Tajti J, Nyari A, et al. Therapeutic approaches for peripheral and Central neuropathic pain. Behav Neurol. 2019;2019: 8685954.
[14] Obici L, Suhr OB. Diagnosis and treatment of gastrointestinal dysfunction in hereditary TTR amyloidosis. Clin Auton Res. 2019;29(Suppl 1):55–63.
[15] Sperry BW, Hanna M, Shah SJ, et al. Spironolactone in patients with an echocardiographic hfpef phenotype suggestive of cardiac amyloidosis: results from TOPCAT. JACC Heart Fail. 2021;9(11):795–802.
[16] Teng C, Li P, Bae JY, et al. Diagnosis and treatment of transthyretin-related amyloidosis cardiomyopathy. Clin Cardiol. 2020;43(11):1223–1231.
[17] Ericzon BG, Wilczek HE, Larsson M, et al. Liver transplantation for hereditary transthyretin amyloidosis: after 20 years still the best therapeutic alternative? Transplantation. 2015; 99(9):1847–1854.
[18] Berk JL, Dyck PJ, Obici L, et al. The diflunisal trial: update on study drug tolerance and disease progression. Amyloid. 2011; 18(sup1):196–192.
[19] Coelho T, Maia LF, Martins da Silva A, et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology. 2012;79(8):785–792.
[20] Merlini G, Plante-Bordeneuve V, Judge DP, et al. Effects of tafamidis on transthyretin stabilization and clinical outcomes in patients with non-Val30Met transthyretin amyloidosis. J Cardiovasc Transl Res. 2013;6(6):1011–1020.
[21] Monteiro C, Mesgazardeh JS, Anselmo J, et al. Predictive model of response to tafamidis in hereditary ATTR polyneuropathy. JCI Insight. 2019;4(12):e126526.
[22] Maurer MS, Schwartz JH, Gundapaneni B, et al. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. N Engl J Med. 2018;379(11):1007–1016.
[23] Monteiro C, Martins da Silva A, Ferreira N, et al. Cerebrospinal fluid and vitreous body exposure to orally administered tafamidis in hereditary ATTRV30M (p.TTRV50M) amyloidosis patients. Amyloid. 2018;25(2): 120–128.
[24] Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med. 2018;379(1):11–21.
[25] Adams D, Polydefkis M, Gonzalez-Duarte A, et al. Long-term safety and efficacy of patisiran for hereditary transthyretin-mediated amyloidosis with polyneuropathy: 12-month results of an open-label extension study. Lancet Neurol. 2021;20(1):49–59.
[26] Solomon SD, Adams D, Kristen A, et al. Effects of patisiran, an RNA interference therapeutic, on cardiac parameters in patients with hereditary transthyretin-mediated amyloidosis. Circulation. 2019;139(4):431–443.
[27] Habtemariam BA, Karsten V, Attarwala H, et al. Single-dose pharmacokinetics and pharmacodynamics of transthyretin targeting n-acetylgalactosamine-small interfering ribonucleic acid conjugate, vutrisiran, in healthy subjects. Clin Pharmacol Ther. 2021;109(2):372–382.
[28] Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen treatment for patients with hereditary transthyretin amyloidosis. N Engl J Med. 2018;379(1):22–31.
[29] Penchala SC, Connelly S, Wang Y, et al. AG10 inhibits amyloidogenesis and cellular toxicity of the familial amyloid cardiomyopathy-associated V122I transthyretin. Proc Natl Acad Sci U S A. 2013;110(24):9992–9997.
[30] Damy T, Garcia-Pavia P, Hanna M, et al. Efficacy and safety of tafamidis doses in the tafamidis in transthyretin

cardiomyopathy clinical trial (ATTR-ACT) and long-term extension study. Eur J Heart Fail. 2021;23(2):277–285.
[31] BridgeBio Pharma Reports Month 12 Topline Results From Phase 3 ATTRibute-CM Study [Internet]; [cited 2021]. Available from: https://www.bridgebio.com/news/bridgebio-pharma-reports-month-12-topline-results-from-phase-3-attribute-cm-study.
[32] Pinheiro F, Varejao N, Esperante S, et al. Tolcapone, a potent aggregation inhibitor for the treatment of familial leptomeningeal amyloidosis. FEBS J. 2021;288(1):310–324.
[33] Gamez J, Salvado M, Reig N, et al. Transthyretin stabilization activity of the catechol-O-methyltransferase inhibitor tolcapone (SOM0226) in hereditary ATTR amyloidosis patients and asymptomatic carriers: proof-of-concept study(.). Amyloid. 2019;26(2):74–84.
[34] Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med. 2021;385(6):493–502.
[35] Hosoi A, Su Y, Torikai M, et al. Novel antibody for the treatment of transthyretin amyloidosis. J Biol Chem. 2016;291(48):25096–25105.
[36] Suhr O, Grogan M, Martins da Silva A, et al. Neurological and cardiac improvements with PRX004 in amyloidosis patients: results of a phase 1 study, 2021 emerging science abstracts. 2021 AAN annual meeting abstracts. Neurology. 2021;96(22):e2783–e2788.
[37] Michalon A, Hagenbuch A, Huy C, et al. A human antibody selective for transthyretin amyloid removes cardiac amyloid through phagocytic immune cells. Nat Commun. 2021;12(1):3142.
[38] Griffin JM, Rosenthal JL, Grodin JL, et al. ATTR amyloidosis: current and emerging management strategies: JACC: CardioOncology state-of-the-Art review. JACC Cardiooncol. 2021;3(4):488–505.
[39] Luzzatto L, Hyry HI, Schieppati A, et al. Outrageous prices of orphan drugs: a call for collaboration. Lancet. 2018;392(10149):791–794.
[40] Aimo A, Rapezzi C, Perfetto F, et al. Quality of life assessment in amyloid transthyretin (ATTR) amyloidosis. Eur J Clin Invest. 2021;51(11):e13598.
[41] Dang D, Fournier P, Cariou E, et al. Gateway and journey of patients with cardiac amyloidosis. ESC Heart Fail. 2020;7(5):2418–2430.
[42] Huda A, Castano A, Niyogi A, et al. A machine learning model for identifying patients at risk for wild-type transthyretin amyloid cardiomyopathy. Nat Commun. 2021;12(1):2725.
[43] Saelices L, Chung K, Lee JH, et al. Amyloid seeding of transthyretin by ex vivo cardiac fibrils and its inhibition. Proc Natl Acad Sci USA. 2018;115(29):E6741–E6750.

Appendix A

Criteria for determining the level of evidence and the grade for recommendation

Level of evidence

I Evidence obtained from meta-analysis of multiple, well-designed, controlled studies
Randomized trials with low false-positive and low false-negative errors (high power)

II Evidence obtained from at least one well-designed experimental study
Randomized trials with high false-positive or false-negative errors (low power)

III Evidence obtained from well-designed, quasi-experimental studies, such as nonrandomized, controlled single-group, pre-post, cohort, time or matched case-control series

IV Evidence from well-designed, nonexperimental studies, such as comparative and correlational descriptive and case studies

V Evidence from case reports and clinical examples

Grade for recommendation

A. There is evidence of type I or consistent findings from multiple studies of types II, III or IV.
B. There is evidence of types II, III or IV and findings are generally consistent.
C. There is evidence of types II, III or IV but findings are inconsistent.
D. There is little or no systematic empirical evidence.