Review Article

Amyloidosis—the Diagnosis and Treatment of an Underdiagnosed Disease

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Summary

Background: Systemic amyloidosis is a multi-system disease caused by fibrillary protein deposition with ensuing dysfunction of the affected organ systems. Its diagnosis is often delayed because the manifestations of the disease are variable and non-specific. Its main forms are light chain (AL) amyloidosis and transthyretin-related ATTR amyloidosis, which, in turn, has both a sporadic subtype (wildtype, ATTRwt) and a hereditary subtype (mutated, ATTRv).

Methods: This review is based on pertinent publications that were retrieved by a selective search in PubMed covering the years 2005 to 2019.

Results: No robust epidemiological figures are available for Germany to date. Both AL amyloidosis and hereditary ATTR amyloidosis are rare diseases, but the prevalence of ATTRwt amyloidosis is markedly underestimated. The diagnostic algorithm is complex and generally requires histological confirmation of the diagnosis. Only cardiac ATTR amyloidosis can be diagnosed non-invasively with bone scintigraphy once a monoclonal gammopathy has been excluded. AL amyloidosis can be considered a complication of a plasma cell dyscrasia and treated with reference to patterns applied in multiple myeloma. Despite the availability of causally directed treatment, it has not yet been possible to reduce the mortality of advanced cardiac AL amyloidosis. Three drugs (tafamidis, patisiran, and inotersen) are now available to treat grade 1 or 2 polyneuropathy in ATTRv amyloidosis, and further agents are now being tested in clinical trials. It is expected that tafamidis will soon be approved in Germany for the treatment of cardiac ATTR amyloidosis.

Conclusion: The diagnosis of amyloidosis is difficult because of its highly varied presentation. In case of clinical suspicion, a rapid, targeted diagnostic evaluation and subsequent initiation of treatment should be performed in a specialized center. When the new drugs to treat amyloidosis become commercially available, their use and effects should be documented in nationwide registries.

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The term systemic amyloidosis embraces a number of heterogeneous syndromes characterized by protein deposits in the form of insoluble fibrils in the patient's tissues (1). The clinical findings vary according to the identity of the protein concerned and the extent and pattern of organ involvement (1, 2). As yet there are no valid epidemiological data for systemic amyloidosis in Germany. Light chain (AL) amyloidosis is so far considered to be the most frequently occurring form (1, 3), with an incidence of 8.9–12.7/million person-years and prevalence of 40–58/million person-years (4). Hereditary transthyretin (ATTRv) amyloidosis is estimated to affect 5000–10 000 persons worldwide (5). These figures meet the definition of a rare disease. In contrast, age-related wild-type transthyretin (ATTRwt) amyloidosis is being diagnosed increasingly often: 25% of patients with heart failure with preserved left ventricular ejection fraction (HFpEF) over 80 and 13% of those over 60 years of age are thought to be affected (6, 7). This means that the prevalence has been underestimated.

This article reviews the data on systemic amyloidosis, focusing on the prognostic relevance of cardiac involvement, on diagnosis of the disease, and on the spectrum of emerging treatment concepts.

Methods

We carried out a selective search of PubMed for pertinent records published in the period 2005–2019. The search terms were “systemic amyloidosis,” “AL amyloidosis,” “ATTR amyloidosis,” “senile systemic amyloidosis,” “cardiac amyloidosis,” “familial amyloid polyneuropathy,” and “familial amyloid cardiomyopathy.”

Pathophysiology

Systemic amyloidosis arises from the formation of insoluble amyloid fibrils, which in turn results from deposition of misfolded proteins. Over 30 proteins are known to be involved (8), causing different subtypes that cannot be distinguished by clinical means.

- **AL amyloidosis:** This results from the deposition of monoclonal free light chains—systemically due to monoclonal gammopathy, multiple myeloma, or, more rarely, B-cell lymphoma, or locally due to local production of light chains. In systemic manifestations, circulating light chains have a direct cardiotoxic action (1). Deposits of light chains lead to mechanical interference and have cytotoxic and proapoptotic effects (1).

TABLE

The clinical manifestations of systemic amyloidosis

Organ	Symptoms
Heart	Dyspnea, peripheral edema, anasarca, pleural effusion, pericardial effusion, palpitations, irregular heart-beat, syncopes, hypotension or regression of arterial hypertension, reduced heart rate variability
Kidney	Edema, foamy urine, proteinuria (to the point of nephrotic syndrome) with predominant albuminuria, renal failure
Liver	Hepatomegaly, elevated liver stiffness, ascites, alkaline phosphatase elevation
Gastrointestinal tract	Dysphagia, loss of appetite, weight loss, nausea, postprandial fullness, meteorism, diarrhea, obstipation, gastrointestinal bleeding
Peripheral and autonomic nervous system	Polyneuropathy (progressive, symmetric, axonal/small fiber, overall very variable), vegetative dysregulation (orthostatic dysregulation), intestinal motility disorder, urinary retention disorder, erectile dysfunction
Eye	Dry eye, vitreous body opacity, glaucoma, retinal angiopathy
Soft tissues and other manifestations	Macroglossia, hoarseness, coagulation disorders, purpura/cutaneous hemorrhage, e.g., periorbital, carpal tunnel syndrome, swollen joints, splenomegaly, myasthenia, fatigue, biceps tendon rupture, lumbar spinal stenosis

The clinical manifestations of systemic amyloidosis are extremely variable. Cardiac involvement relevant to the prognosis is seen particularly in AL and ATTR amyloidosis.

• **ATTR amyloidosis:** The causal protein is transthyretin (TTR), the transport protein of thyroxine and retinol-binding protein/vitamin A (9). Although the underlying mechanism has not been fully elicited (10), the essential feature seems to be mechanical/enzymatic cleavage of fragments from the TTR tetramer by proteases. This leads to destabilization and misfolding of the monomers with tissue deposition triggered by C-terminal fragments (10). Furthermore, amyloidogenic *TTR* mutations facilitate the deposition process in ATTRv amyloidosis by increasing thermodynamic instability (11). Besides mutant TTR, the deposits in patients with ATTRv amyloidosis also contain wild-type TTR (12). Altogether, more than 120 causal mutations have been identified, typically inherited in an autosomal dominant fashion with variable penetrance. Analogously, natural TTR is co-deposited in ATTRwt amyloidosis (9).

Clinical findings

The clinical manifestations of amyloidosis vary widely depending on the subtype and on the pattern and severity of organ involvement (*Table*). Owing to low specificity, prodromes are frequently misinterpreted—typically as symptoms of a common illness. Diagnosis is often delayed: 20% of patients with AL amyloidosis are not correctly diagnosed until 2 years or longer after the first symptoms, and in 42% of those with cardiac ATTRwt amyloidosis the diagnostic process takes more than 4 years (13, 14). Findings that should serve as “red flags” for continued diagnostic efforts include nephrotic syndrome, HFpEF, rapidly progressive polyneuropathy, unexplained hepatomegaly or diarrhea, unexplained weight loss, and otherwise inexplicable elevation of cardiac biomarkers in plasma cell dyscrasia (3).

The manifestations of AL amyloidosis are primarily cardiac (about 75–80%) and renal (about 65%); less frequently, the soft tissues (15%), the liver (15%), the nervous system (10%), and the gastrointestinal tract (5%) are involved. Cardiac involvement worsens the prognosis (1). Typically, AL amyloidosis progresses rapidly and thus demands immediate diagnosis and treatment. Around 30% of patients diagnosed with advanced cardiac amyloidosis die within one year, and so far the effective new treatment options have not decreased this early mortality (1). The 4-year survival rate varies between 40% and 60% (1).

ATTRwt amyloidosis frequently presents with a cardiac phenotype in the form of slowly progressing HFpEF (15). There is often accompanying neurological involvement, e.g., symmetric, extremely variable sensorimotor polyneuropathy, but purely neurological manifestations are rare (4%). ATTRwt amyloidosis is known to be associated with carpal tunnel syndrome and lumbar spinal stenosis. Men are predominantly affected. The median duration of survival after diagnosis is about 4 years (15).

Depending on the mutation, the phenotype of ATTRv amyloidosis is predominantly cardiac, neuropathic, or mixed cardiac/neuropathic (16). Much more infrequently, the kidney, gastrointestinal tract, eye, leptomeninges/meninges, or vascular system (amyloidangiopathy) are involved.

Diagnosis

The diagnosis of amyloidosis is a multi-stage process that should take place without delay (10).

Histological confirmation of suspected amyloidosis

Histological demonstration of amyloidosis is essential for confirmation of the diagnosis. A suitable

FIGURE 1

Diagnostic algorithm. The first step is to establish whether or not monoclonal gammopathy is present. If monoclonal gammopathy is absent and skeletal scintigraphy is positive (cardiac tracer uptake grade 2–3), cardiac ATTR amyloidosis is confirmed (19); TTR mutation analysis is recommended. If scintigraphy is negative (grade 0–1), biopsy including amyloid subtyping should ensue, accompanied if indicated by genetic analysis for rarer forms (19).

If monoclonal gammopathy is present, histology including subtyping is indispensable. Painstaking characterization of organ involvement is essential before commencement of treatment. Green arrow: "if positive, proceed to;" red arrow: "if negative, proceed to."

*1 The diagnostic sensitivity of abdominal fat biopsy is 84% for cardiac AL amyloidosis, 15% for ATTRwt amyloidosis, and 45% for ATTRv amyloidosis (depending on the underlying TTR mutation); for salivary gland biopsy after negative abdominal fat biopsy, sensitivity is 58%, specificity 100%, and negative predictive value 91%.

*2 Suitable tracers are ^{99m}Tc -DPD, ^{99m}Tc -PYP, and ^{99m}Tc -HMDP

*3 Definitive exclusion only possible by organ biopsy; NB: risk of false-negative biopsy

*4 ATTRv and ATTRwt amyloidosis are differentiated by means of TTR mutation analysis

*5 NB: Risk of false-negative biopsy

NT-proBNP, N-Terminal pro-hormone brain-natriuretic peptide; TTR, transthyretin

low-invasive procedure is aspiration of abdominal fat, the sensitivity of which depends on the subtype of amyloidosis concerned (84% for cardiac AL amyloidosis, 15% for cardiac ATTRwt amyloidosis, 45% for cardiac ATTRv amyloidosis (17). If the result is negative, salivary gland biopsy should follow (18). Direct organ biopsy should be resorted to only if the diagnosis remains uncertain or in the presence of a constellation such as isolated cardiac involvement with coexisting monoclonal gammopathy.

Cardiac ATTR amyloidosis in the absence of monoclonal gammopathy (negative immunofixation from serum and 24-h urine together with normal levels of free light chains) is the only subtype amenable to noninvasive diagnosis by means of skeletal scintigraphy (sensitivity > 99%, specificity 86%) (19).

Suitable tracers are ^{99m}Tc -DPD, ^{99m}Tc -PYP, and ^{99m}Tc -HMDP (10). Cardiac involvement in ApoAI and AA amyloidosis might result in positive scintigraphy as well (10).

Amyloid subtyping and mutation analysis

Amyloid subtyping from a tissue sample obtained by biopsy is obligatory, particularly because the presence of a monoclonal gammopathy does not prove the existence of AL amyloidosis: in 20% of cases, ATTR amyloidosis is accompanied by monoclonal gammopathy (19). The subtyping can be achieved by means of mass spectroscopy, immunohistochemistry (NB: higher error rate), or immunoelectron microscopy in a special laboratory with suitably experienced personnel. Should a potentially hereditary form of amyloidosis be demonstrated, the corresponding gene must be analyzed for mutations.

FIGURE 2

Treatment algorithm for systemic AL amyloidosis. The treatment of AL amyloidosis requires strict risk stratification, which in turn necessitates precise characterization of organ involvement. The classic protocols for standard chemotherapy are CyBorD (bortezomib, cyclophosphamide, dexamethasone) and BMDex (bortezomib, melphalan, dexamethasone).

*Depending on the “fragility” of the patient, individual dose adjustments may be necessary. For instance, fluid retention due to steroid administration in advanced cardiac amyloidosis may become problematic, requiring dose reduction or even complete discontinuation of steroids.

BM, Bone marrow; CRAB, hypercalcemia, renal insufficiency, anemia, and bone lesions; CyBorD, bortezomib, cyclophosphamide, dexamethasone; ECOG performance status, performance status according to Eastern Co-operative Oncology Group; eGFR, estimated glomerular filtration rate; immunomodulators (e.g. lenalidomide [Revlimid], pomalidomide); NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA stage, stage according to the classification of the New York Heart Association; VGPR, very good partial remission

Characterization of organ involvement

Precise characterization of the extent and severity of organ involvement is essential, especially for treatment planning in cases of AL amyloidosis (1). The diagnosis of cardiac involvement in a patient with extracardiac detection of amyloid rests on otherwise inexplicable elevation of the cardiac biomarkers N-terminal prohormone brain-natriuretic peptide (NT-proBNP) and troponin, together with characteristic morphological features on diagnostic imaging. The grading of cardiac involvement is based primarily on NT-proBNP and troponin (1, 2, 10, 15). Renal manifestations are marked by protein-

uria with predominant albuminuria, and impairment of renal function. Isolated elevation of alkaline phosphatase and hepatomegaly point to involvement of the liver.

Figure 1 shows a detailed diagnostic algorithm for patients suspected to have cardiac amyloidosis. The order in which investigations are carried out and the appraisal of the findings is complex, so the diagnostic investigations should take place at a specialized center. In particular, such a facility should be contacted at the first suspicion of cardiac AL amyloidosis owing to the prognostic import of heart involvement.

Treatment

AL amyloidosis

In principle, AL amyloidosis can be understood as a complication of plasma cell dyscrasia. It is treated with reference to treatment patterns applied to multiple myeloma, i.e. targeting rapid elimination of the amyloidogenic, (cardio)toxic light chains.

Patients can be risk-stratified into “fit” and “fragile” on the basis of clearly defined parameters of suitability for high-dose chemotherapy (Figure 2).

“Fit” patients (10–25%) should receive high-dose chemotherapy. Induction chemotherapy is necessary only if the initial plasma cell infiltration of bone marrow is >10% or the CRAB criteria (hypercalcemia, renal insufficiency, anemia, bone lesions) are fulfilled. Usually, a single administration of high-dose chemotherapy follows stem-cell mobilization with granulocyte colony-stimulating factor (G-CSF) without chemotherapy beforehand. Particularly patients with known translocation *t(11;14)* profit from the high-dose chemotherapy concept/high-dose melphalan, while a poorer response has been reported for bortezomib-based protocols (20, 21).

A proteasome inhibitor-based regimen has become established for “fragile” patients. For younger patients, CyBORd (bortezomib, cyclophosphamide, dexamethasone) should be preferred to BMDex (bortezomib, melphalan, dexamethasone) owing to the stem cell toxicity of melphalan, in order to retain the option of stem cell apheresis and high-dose chemotherapy at a later date (1). MDex is an effective treatment option in polyneuropathy and gain of chromosome 1q21 (1, 22).

Patients who do not respond adequately to their first-line treatment should be switched to daratumumab, a monoclonal anti-CD38 antibody.

In the event of renewed or increasing disease activity after completion of the first-line treatment, the latter can be repeated. Alternatively, immunomodulator (IMiD)-based protocols (particularly lenalidomide [Revlimid] and pomalidomide) or daratumumab can be used (3). The recently introduced proteasome inhibitors such as ixazomib may represent an alternative (23). Further therapeutic agents are in clinical testing.

Dose-reduced (standard) protocols may be necessary in very advanced disease. In young patients, organ transplantation before initiation of chemotherapy can be considered in order to render them amenable to treatment.

In AL amyloidosis, a hematological response is distinguished from a response at organ level (Figure 3). Hematological response means a decrease in serological activity corresponding to a decrease in the free light chain difference; ideally, negative immunofixation in blood and (24-h) urine with normal levels of free light chains in serum. Organ response means functional improvement of the organs involved, which may in some cases be delayed until a number of months after the onset of hematological response (24). The treatment response should always be

FIGURE 3

Assessment of treatment response in systemic AL amyloidosis

Two qualities of treatment response can be distinguished: hematological and organ response. The hematological response is defined either by the reduction in light chains and by the decrease in the difference between involved and non-involved free light chains (dFLC), respectively. The organ response is determined individually for each organ involved, because this is highly relevant for the prognosis, and is judged primarily on the basis of laboratory findings (39). A low initial dFLC (<50 mg/L) is associated with a good prognosis and requires adjustment of the criteria for assessment of the treatment response (40).

eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA stage, stage according to the classification of the New York Heart Association

assessed every two to three cycles and, if required, the treatment adjusted. Treatment can be ended two cycles after the peak response (1).

If the response is inadequate, the treatment must be modified immediately.

Hereditary ATTR amyloidosis

For many years the only treatment for ATTRv amyloidosis was liver transplantation (25), but this is no longer the case. The 20-year survival rate after liver transplantation was 55% in an international analysis of 1940 patients with ATTRv amyloidosis from 19 countries (25). Important for the prognosis is timely recognition of the indication for transplantation and the absence of severe organ-related manifestations at the time of surgery (26). The fully functional explanted liver of a patient with ATTRv amyloidosis can be donated to another patient who would otherwise not receive a replacement organ (domino transplantation) (10). However, the transplant recipient runs the risk of iatrogenic ATTRv amyloidosis (10).

The primary aim of more recently introduced treatments is to slow down or halt disease progression by means of gene silencing strategies, TTR stabilization, and depletion of existing amyloid deposits (Figure 4). A detailed overview of selected studies, some published and others still recruiting patients, can be found in the eTable.

Gene silencers inhibit hepatic synthesis of the causal TTR protein by mRNA interference (eBox). Only patisiran and inotersen have so far been approved for use in patients who have ATTRv

FIGURE 4

Treatment approaches for ATTR amyloidosis. Liver transplantation is decreasing in importance because of its invasiveness, the scarcity of organs, and the danger of disease progression due to deposition of wild-type transthyretin onto existing deposits. Alternative treatments, on the other hand, have been gaining in importance. TTR stabilizers inhibit dissociation of the transthyretin tetramer into monomers and dimers and thus prevent their deposition as amyloid fibrils. To date, only the TTR stabilizer tafamidis is approved in Germany for use against ATTRv amyloidosis with grade I neurological manifestations. Gene silencers such as inotersen and patisiran suppress the hepatic synthesis of transthyretin through mRNA interference and are licensed in Germany for use in ATTRv patients with grade I and II polyneuropathy. All other substances are currently in clinical testing. The approved substances are written in bold type.

amyloidosis with polyneuropathy grade I or II, while no agents have yet been licensed for cardiac involvement.

Patisiran, an siRNA, was investigated in the randomized, double-blinded, placebo-controlled APOLLO trial (phase III) of 225 ATTRv amyloidosis patients with polyneuropathy. After 18 months, the verum group showed improvement of 6.0 ± 0.7 points in the modified Neuropathy Impairment Score (mNIS + 7), the primary endpoint, while the control group score worsened by 28.0 ± 2.6 points ($p < 0.001$) (27). Secondary endpoints were also more favorable in the patisiran group, for example quality of life (Norfolk QoL-DN; difference -21.1 points; $p < 0.001$), speed in the 10-m walking test (difference $+0.31$ m/s; $p < 0.001$), and nutritional status (modified BMI; difference $+116$; $p < 0.001$). In the echocardiographic substudy (56% of the study population), patients who were given patisiran showed a consistent decrease in mean wall thickness (difference 0.9 mm, $p = 0.017$); signs of increased end-diastolic volume and stroke volume; and a decrease in global longitudinal strain. Heart-related hospitalizations and overall mortality were lower with patisiran than with placebo (18.7 versus 10.1 events per 100 patient-years) (28). Patisiran is administered intravenously every 3 weeks. Infusion reactions constitute the principal adverse effect.

Inotersen, an antisense oligonucleotide, was investigated in 172 patients in the phase-III NEURO-TTR study. With regard to the primary endpoints, patients who were given inotersen showed slower disease pro-

gression as measured by the mNIS + 7 (difference -19.7 points; $p < 0.001$) and better development of quality of life (difference in Norfolk QoL-DN score -11.7 points; $p < 0.001$) (29). Inotersen is administered subcutaneously once a week. The clinically relevant adverse effects are glomerulonephritis (3 %) and thrombopenia (3 %), necessitating regular laboratory monitoring.

The multicenter, double-blind, placebo-controlled phase-III trial of revusiran in patients with manifestations of cardiac ATTRv amyloidosis showed increased overall and cardiovascular mortality in the revusiran arm.

Other newly developed therapeutics are long-acting subcutaneously administered substances: Vutrisiran is a so-called enhanced stability chemistry (ESC) GalNAc conjugate with better hepatic uptake which, compared with patisiran, promises greater efficacy and better stability despite a lower administration volume.

The randomized phase-III trial HELIOS-A is comparing the efficacy and safety of vutrisiran (25 mg subcutaneously every 3 months) and patisiran in ATTRv patients with neurological manifestations (NCT03759379). The complementary phase-III trial HELIOS-B for cardiac manifestations is at the recruitment stage (NCT04153149).

AKCEA-TTR-LRx (ION-682884) is a GalNAc₃ antisense oligonucleotide conjugate with greater stability than inotersen, better hepatic uptake, and higher efficacy, enabling monthly subcutaneous administration. The phase-III trials on neurological and

cardiac manifestations (NEURO-TTRansform [NCT04136184] and CARDIO-TTRansform [NCT04136171], respectively) are currently recruiting.

Stabilization of the transthyretin tetramer can be achieved particularly with tafamidis (30), diflunisal (31), AG-10 (32), and tolcapon (33). The only TTR stabilizer licensed for use in Germany is tafamidis for ATTRv patients with grade I polyneuropathy; approval for cardiac ATTRv amyloidosis is expected in 2020. Tafamidis occupies the thyroxine binding site, thus preventing TTR tetramer dissociation. It is given orally and its primary effect is to slow the progress of the disease (34, 35). Administration at an early stage in the disease course is crucial. In the randomized, double-blind, phase-III ATTR-ACT trial on patients with cardiac ATTR amyloidosis (ATTRwt and ATTRv), tafamidis significantly decreased the overall mortality (hazard ratio 0.70; 95% confidence interval [0.51; 0.96]) and the number of cardiovascular-related hospitalizations (0.48 vs. 0.70/year) (36). The adverse effects were comparable with those in the placebo group (36). Diflunisal was found to slow the progression of neurological manifestations compared with placebo (increase of 25 points in NIS + 7 score; difference 16 points, $p < 0.001$). The data regarding the efficacy of diflunisal in ATTR cardiomyopathy come from a small Japanese study ($n = 40$, ATTRv amyloidosis, 24 months' observation) which showed signs of stabilization of cardiac wall thickness.

A pilot study of another new TTR stabilizer, tolcapon, has recently shown TTR stabilization in all participants (NCT02191826), but phase-III data are lacking. Because tolcapon penetrates the blood–brain barrier, its short-term TTR-stabilizing effects have been investigated in an early phase-I study in patients with symptomatic and asymptomatic leptomeningeal involvement; however, the results have not yet been published (NCT03591757).

Doxycycline/tauroursodeoxycholic acid (TUDCA) targets acceleration of fibril degeneration and fibril resorption (37). The studies conducted to date have small case numbers and suggest a positive effect in patients with cardiac involvement. A larger randomized, placebo-controlled phase-III trial in patients with ATTRwt and ATTRv amyloidosis is now recruiting (NCT03481972). Other substances currently being tested include the monoclonal antibody PRX004, directed against monomers and deposited TTR amyloid (38).

The potential significance of the novel treatments is not yet clear—no studies comparing them directly have been published. According to an expert recommendation, the primary use of gene silencers is worthwhile particularly in aggressive disease. It is important to perform genetic testing of potential carriers of mutations (*eMethods*) and to initiate treatment as soon as the first manifestations are noted. The mean annual cost of treatment is around € 160 000 for

Key Messages

- Variability of the phenotype, the absence of specific early symptoms, and the perceived lack of treatment options are responsible for the typical delay in the diagnosis of amyloidosis.
- Nephrotic syndrome, cardiac insufficiency with preserved ejection function (HFpEF), unexplained elevation of cardiac biomarkers in plasma cell dyscrasia, rapidly progressive polyneuropathy, unexplained hepatomegaly or diarrhea may indicate the presence of amyloidosis.
- Histological demonstration of amyloid, including subtyping, is indispensable for diagnosis. The sole exception is scintigraphic detection of cardiac transthyretin (ATTR) amyloidosis in the absence of monoclonal gammopathy.
- Early initiation of treatment improves the prognosis of both light-chain amyloidosis and ATTR amyloidosis, provided a licensed therapeutic is available for the patient's pattern of organ involvement.
- Cooperation with specialized interdisciplinary centers is essential.

tafamidis, € 320 000 for inotersen, and € 360 000 for patisiran. In-label use in Germany is covered by health insurance.

Wild-type ATTR amyloidosis

No substance is yet approved for the treatment of ATTRwt amyloidosis. The efficacy of tafamidis against cardiac ATTRwt amyloidosis has been demonstrated (36). Licensing in Germany is anticipated, but until that time only off-label use is possible.

Conflict of interest statement

Dr. Ihne's research was supported by the Comprehensive Heart Failure Center (CHFC) Würzburg and the Interdisciplinary Center of Clinical Research (IZKF), Würzburg. She has received funding for a project of her own initiation from Akcea; consultancy and lecture fees from Takeda, Pfizer, Janssen, and Akcea; and reimbursement of congress registration fees as well as travel and accommodation costs from Takeda, Pfizer, Akcea, and Alnylam. An internship abroad was supported by ONLUS.

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Prof. Sommer is a member of the advisory boards of Akcea, Alnylam, and Pfizer. She has received payments for the preparation of scientific meetings from Alnylam and Pfizer, and has received funding for a project of her own initiation from Pfizer.

Prof. Knop is a member of the advisory boards of Celgene, Amgen, Bristol-Myers Squibb, and Molecular Partners.

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Prof. Geier is a member of the steering committees of Gilead, Intercept, and Novartis. He receives consultancy fees from AbbVie, Alexion, BMS, Gilead, Intercept, Ipsen, Novartis, Pfizer, and Sequana, and has received lecture fees from AbbVie, Alexion, BMS, CSL Behring, Falk, Gilead, Intercept, Merz, Novartis, and Sequana.

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► Supplementary material

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eBOX

mRNA interference

Messenger RNA (mRNA) contains a transcript of a gene segment. Normally it transports this information from the cell nucleus to the ribosomes, so that, with the aid of the transcript, the corresponding proteins can be formed.

mRNA interference occurs when short segments of RNA (e.g., siRNA or oligonucleotides) interact with autologous RNA and various protein complexes. This destroys the mRNAs, preventing formation of the corresponding proteins.

eMETHODS

Presymptomatic genetic testing and follow-up of persons with TTR mutations

A decisive role is played by early detection of developing symptoms of amyloidosis in carriers of amyloidogenic mutations, because there are no preventive interventions and the available treatments are most effective in the early stages of the disease (e1). Presymptomatic genetic testing should be offered to the relatives of patients with ATTRv amyloidosis. Especially important in this regard is diagnostic investigation of siblings at the age where disease onset can be anticipated. Particular care should be taken in deciding the timing of testing in cases where a greater elapse of time before disease onset seems likely. Factors such as negative impacts of knowledge of the mutation on quality of life and psychological wellbeing should not be underestimated. The German law on genetic diagnosis stipulates that advice should be provided by a human geneticist or a physician with subject-linked permission for genetic counselling.

Following a recently issued international expert recommendation, the first step is to define the expected time of disease onset, based on the exact nature of the mutation and the onset of disease in the index patient (e2). Monitoring should begin with an exhaustive basic work-up 10 years before this time, followed by annual visits. The schedule must be adjusted according to the anticipated aggressiveness of the disease. Equally, the specific investigations performed depend on anticipated disease phenotype (e2).

Fulfillment of minimal criteria for the diagnosis of ATTR amyloidosis in known carriers of TTR mutations should trigger initiation of treatment (e2): an

objective symptom or clinical correlate thereof that is definitively associated with the onset of ATTR amyloidosis (sensorimotor neuropathy [changes relative to baseline], autonomic neuropathy or neuronal/sexual dysfunction, cardiac involvement, renal or ocular involvement) or a symptom that is probably associated with disease onset despite absence of identifiable clinical signs together with an abnormal test result or two abnormal test results without subjective symptoms.

Supportive treatment

Optimal fluid management with primary use of diuretics is vital, whereas conventional cardiac insufficiency treatment in the absence of evidence is of secondary importance. Calcium-channel blockers are contra-indicated owing to their negative inotropic action and potential interaction with the amyloid fibrils (e3, e4).

In the presence of symptomatic bradycardia or marked chronotropic incompetence the use of a cardiac pacemaker may be worthwhile. Insertion of an implantable cardioverter-defibrillator (ICD) can be considered in a patient with malignant cardiac arrhythmia. However, it has not yet been shown that prophylactic ICD implantation prolongs long-term survival (e5).

Symptomatic hypotension as a consequence of autonomic nervous system involvement may necessitate the administration of midodrine and/or fludrocortisone together with physical measures such as compression treatment (e6). Motility-enhancing or -inhibiting drugs can be given to treat gastrointestinal symptoms. Adequate calorie intake is essential.

eTABLE

Treatment approaches and selected study results in ATTR amyloidosis

Sub- stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status
Tafamidis	Coelho et al. 2012 (NCT00409175) (35)	Phase II/III, multicenter, double-blind, placebo-controlled, 1:1 randomization (tafamidis 20 mg/d vs. placebo) 128 pat. (18–75 y) with early-stage neurological manifestation of ATTR amyloidosis and positivity for Val30Met mutation – n = 162 pat. screened – n = 128 pat. randomized (n = 65 tafamidis, n = 63 placebo) – 13 pat./arm received LTx – n = 19 drop-outs before 12 months – Intention-to-treat population (ITT) n = 125 – Population analyzable regarding efficacy (EE) n = 87 – Observation period 18 months	Primary: 1. Percentage of responders measured using NIS-LL at 18 months; response: improvement or stabilization (change by <2 points compared with baseline) 2. Changes in Norfolk QOL-DN Total Quality of Life (TQOL) score at 18 months compared with baseline Secondary: 1. Changes in NIS-LL at 6, 12, 18 months compared with baseline 2. Percentage of responders measured using NIS-LL at 6 and 12 months 3. Changes in Norfolk QOL-DN Total Quality of Life (TQOL) score at 6 and 12 months compared with baseline 4. Changes in Norfolk QOL-DN Domain score at 6, 12 and 18 months compared with baseline 5. Change in Summated 7 score to determine the function of large nerve fibers at 6, 12 and 18 months compared with baseline 6. Change in NTSFNds (small fibers) at 6, 12 and 18 months compared with baseline 7. Change in mBML at 6, 12, 18 months compared with baseline 8. Percentage of pat. with stabilized TTR (at 8 weeks, 6, 12, 18 months)	Evaluation of tafamidis vs. placebo – Intention-to-treat analysis: NIS-LL response rate 45.3% vs 29.5%, p = 0.068, NNT 6.3; TQOL 2.0 vs 7.2; p = 0.116 – Per-protocol analysis: NIS-LL response rate 60.0% vs 38.1%, p = 0.041; TQOL 0.1 vs 8.9; p = 0.045 – Secondary endpoints predominantly positive – TTR stabilization in 98% vs. 0%; p < 0.0001	AEs with tafamidis similar to placebo – Interruption of study medication 6.2% vs. 4.8% – SAE 9.2% vs. 7.9% – Urinary tract infections in 2 pat. (SAE) in tafamidis arm, no other AE in more than 1 pat. – Death due to LTx-associated complications in 2 pat. (tafamidis) vs. 3 pat. (placebo)	Licensed in Germany for pat. with grade I polyneuropathy in ATTRv amyloidosis
	Coelho et al. 2013 (NCT00791492) (34)	Phase II/III, open-label extension study Neurological manifestation of ATTR amyloidosis with evidence of Val30Met mutation – n = 86 pat. (tafamidis–tafamidis n = 44; placebo–tafamidis n = 41) – 1 pat. received no treatment – Observation period 12 months	Primary: 1. Percentage of responders measured using NIS-LL at 6 months 2. Percentage of responders measured using NIS-LL at 12 months 3. Changes in Norfolk QOL-DN Total Quality of Life (TQOL) score at 6 months compared with baseline 4. Changes in Norfolk QOL-DN Total Quality of Life (TQOL) score at 12 months compared with baseline Secondary: 1. Changes in NIS-LL at 6 and 12 months compared with baseline 2. Changes in Norfolk QOL-DN Domain score at 6 and 12 months compared with baseline 3. Change in Summated 7 score to determine the function of large nerve fibers at 6 and 12 months compared with baseline 4. Change in NTSFNds (small fibers) at 6 and 12 months compared with baseline 5. Change in mBML at 6 and 12 months compared with baseline 6. Change in troponin I concentration at 6 weeks, 3, 6, 12 months compared with baseline 7. Change in NT-proBNP concentration at 6 weeks, 3, 6, 12 months compared with baseline 8. Intraepidermal nerve fiber density (baseline) 9. Percentage of pat. with stabilized TTR (6 and 12 months)	Evaluation of tafamidis initially vs. placebo initially – Group with initial verum: stable rate of change from NIS-LL 0.08 ► 0.11 months (p = 0.60) and TQOL –0.03 ► 0.25 (p = 0.16) – Group with initial placebo: rate of change from NIS-LL 0.34 ► 0.16/month (p = 0.01), TQOL score 0.61 ► –0.16 (p < 0.001) – Pat. with 30 months' tafamidis treatment had better preservation of neurological function (NIS-LL) (55.9%) – TTR stabilization in 94.1% of pat. with 30 months' tafamidis treatment (vs. 93.3% in placebo–tafamidis group)	Similar incidence of AEs and SAEs as for placebo, no treatment discontinuations due to AEs	Licensed in Germany for pat. with grade I polyneuropathy in ATTRv amyloidosis; so far only in Japan and USA for ATTR-related cardiomyopathy; licensing in Germany expected in 2020

Sub- stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status	
Tafamidis	ATTR-ACT, Maurer et al. 2018 (NCT01994889) (36)	Phase III, multicenter, double-blind, placebo-controlled, 2 : 1 : 2 randomization (tafamidis 80 mg/d vs. tafamidis 20 mg/d vs. placebo)	441 pat. (18–90 y) with cardiac manifestation of ATTRwt or ATTRv amyloidosis (n = 106) – Tafamidis 20/80 mg: n = 264 (n = 63 ATTRv, n = 201 ATTRwt) – Placebo: n = 177 (n = 43 ATTRv, n = 134 ATTRwt) – Observation period 30 months	Primary: Combination of overall mortality and incidence of cardiovascular-related hospitalization (baseline vs. 30 months) Secondary (baseline vs. 30 months): 1. Overall mortality 2. Incidence of cardiovascular-related hospitalization 3. Change in walking distance in 6-min walking test 4. Change in KCCQ overall score 5. Cardiovascular-related mortality 6. Percentage of pat. with stabilized TTR at 1 month	Evaluation of tafamidis pooled vs. placebo: – Overall mortality (all-cause): 78 of 264 (29.5%) vs. 76 of 177 (42.9%); hazard ratio 0.70 [0.51; 0.96] – Frequency of cardiovascular hospitalization 0.48 vs. 0.70/year, relative risk ratio 0.68; [0.56; 0.81] – At 30 months, less deterioration in 6-min walking test: 75.7 m [standard error ± 9.2; p <0.001]; first differences at 6 months – At 30 months, less deterioration in KCCQ overall score: 13.7 [standard error ± 2.1; p <0.001]; first differences at 6 months	Safety profile comparable between tafamidis and placebo, previously described increased frequency of diarrhea and urogenital infections not confirmed	Licensed in Germany for pat. with grade I polyneuropathy in ATTRv amyloidosis; so far only in Japan and USA for ATTR-related cardiomyopathy; licensing in Germany expected in 2020
Tolcapone	Gamez et al. 2019 (NCT02191826) (e12)	Phase IIa (proof-of-concept study)	n = 17 – ATTRwt: n = 6; ATTRv (Val30Met): n = 11 – Phase A: single dose 200 mg tolcapone; phase B: 3 × 100 mg tolcapone at 4-h intervals	Primary: TTR stabilization Secondary: 1. Pharmacodynamics (24–32 h) 2. Safety (24 h)	– TTR stabilization in all participants – 2 h after administration: TTR stabilization of at least 20% in 82% of pat. from phase A, 93% of pat. from phase B – Phase A: 52% increase in TTR stabilization 2 h after administration with decrease to 23% after 8 h – Phase B: Increase to 39% 2 h after first administration; preserved at 10 h, decrease at 24 h	No SAEs	Not licensed
No phase-III trials published							

Sub- stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status
AG-10	Judge et al. 2019 (NCT03458130) (e13) Phase II, randomized, double-blind, placebo-controlled (AG-10 vs. placebo)	Cardiomyopathy in ATTRv/wt amyloidosis – n = 49 – Observation period 28 days	Primary: Safety and tolerance (AEs) Secondary: Pharmacokinetics and pharmacodynamics	Good tolerance, almost complete stabilization		Not licensed
	ATTRIBUTE-CM (NCT03860935) Phase III, randomized, double-blind, placebo-controlled (AG-10 vs. placebo)	Cardiomyopathy in ATTRv/wt amyloidosis – 510 participants planned	Primary: Walking distance in 6-min walking test at 12 months Overall mortality and frequency of cardiovascular-related hospitalization at 30 months Secondary: 1. Change in KCCQ overall sum scores at 12 months compared with baseline 2. Change in 6-min walking test at 30 months compared with baseline 3. Change in KCCQ overall sum scores at 30 months compared with baseline 4. Incidence of treatment-associated events (SAE, AEs) within 12 months 5. Incidence of treatment-associated events (SAE, AEs) within 30 months 6. Overall mortality at 30 months 7. Incidence of cardiovascular-related hospitalization within 30 months 8. Cardiovascular-related mortality at 30 months 9. Diverse pharmacodynamic parameters	Study currently recruiting	To be reported	Not licensed
Diflunisal	Berk et al. 2013 (NCT00294671) (e7) Phase III, double-blind, placebo-controlled, 1:1 randomization (diflunisal 2 × 250 mg vs. placebo)	Pat. with neuronal manifestation of biopsy-confirmed ATTRv amyloidosis (18–75 y) – 130 pat. (n = 64 diflunisal, n = 66 placebo) – Observation period 2 y	Primary: Difference in progression of polyneuropathy between treatments, documented by means of NIS+7 (baseline, at 1 and 2 y) Secondary: 1. Changes in Kumamoto Neurologic Scale compared with baseline (1 and 2 y) 2. mBMI (baseline, at 1 and 2 y) 3. SF-36 Physical Component Score (baseline, at 1 and 2 y) 4. SF-36 Mental Component Score (baseline, at 1 and 2 y)	Placebo vs. diflunisal – NIS + 7 score increase by 25.0 [18. 4; 31.6] vs. 8.7 [3.3; 14.1] points, difference 16.3 [8.1; 24.5] points (p < 0.001). – Mean SF-36 Physical Scores decreased by 4.9 [–7.6; –2.2] points with placebo and rose by 1.5 [–0.8; 3.7] points with diflunisal (p < 0.001) – Mean SF-36-Mental-Score fell by 1.1 [–4.3; 2.0] points with placebo, rose by 3.7 [1.0; 6.4] points with diflunisal (p = 0.02). – 29.7% of pat. with diflunisal and 9.4% with placebo showed neurological stabilization at 2 y (<2-point increase in NIS + 7 score; p = 0.007)	Incidence of gastrointestinal, renal, cardiac and hematological AEs similar to placebo; incidence of musculoskeletal and general AE higher with diflunisal, no higher rate of SAEs, 4 pat. discontinued treatment due to diflunisal-associated AE (gastrointestinal bleeding, cardiac decompensation, glaucoma, nausea) vs. 2 pat. in placebo group (headache, renal failure)	Not licensed

Sub- stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status
Patisiran APOLLO, Adams et al. (2018) NCT01969348) (27)	Phase III, multicenter, double-blind, placebo-controlled, 2:1 randomization (patisiran 0.3 mg/kg vs. placebo every 3 weeks)	Pat. (18–85 y) with neuronal manifestation of ATTRv amyloidosis n = 225, cardiac involvement in n = 126 (56%) – Patisiran n = 148 – Placebo n = 77 – Observation period 18 months	Primary: mNIS + 7 Secondary (changes baseline vs. 18 months): 1. Norfolk QoL-DN Questionnaire 2. Neurological Impairment Score–Weakness (NIS-W) 3. R-ODS score 4. 10-min walking test 5. mBMI 6. Autonomic Symptoms Questionnaire (Composite Autonomic Symptom Score [COMPASS 31])	Evaluation of patisiran vs. placebo (baseline vs. 18 months): – mNIS + 7 change -6.0 ± 1.7 vs. 28.0 ± 2.6 (difference -34.0 points; $p < 0.001$) – Change in Norfolk-QoL-DN: -6.7 ± 1.8 vs. 14.4 ± 2.7 (difference -21.1 points; $p < 0.001$) – Change in walking speed in 10-min walking test: 0.08 ± 0.02 m/sec vs. -0.24 ± 0.04 m/sec (difference 0.31 m/sec; $p < 0.001$) – Change in mBMI: -3.7 ± 9.6 vs. -119.4 ± 14.5 (difference 115.7 ; $p < 0.001$)	Overall incidence and type of AEs similar for patisiran and placebo, but 20% mild to moderate infusion reactions in patisiran arm vs. 10% in placebo arm	Licensed in Germany for pat. with grade I–II polyneuropathy in ATTRv amyloidosis
Revusiran ENDEAVOUR (NCT02319005)	Phase II, multicenter, double-blind, placebo-controlled (revusiran vs. placebo)	Pat. (18–90 y) with ATTRv cardiomyopathy, n = 206 – Revusiran n = 140 – Placebo n = 66 – Observation period 18 months	Primary: 1. Distance in 6-min walking test 2. TTR serum level Secondary (changes baseline vs. 18 months): 1. Combination of cardiovascular mortality and cardiovascular-related hospitalization 2. NYHA class 3. KCCQ score 4. Cardiovascular mortality 5. Cardiovascular-related hospitalization 6. Overall mortality		Higher mortality in revusiran arm	Not licensed
Vutrisiran HELIOS-A (NCT03759379)	Phase III, multicenter, randomized, open-label, 3:1 randomization (vutrisiran 25 mg s.c. every 3 months vs. patisiran 0.3 mg/kg every 3 weeks)	ATTRv pat. with neurological involvement – n = 160 planned – Observation period 18 months	Primary: 1. Changes in mNIS + 7 at 9 months compared with baseline 2. Changes in Norfolk QoL-DN overall score at 9 months compared with baseline Secondary: 1. Changes in 10-min walking test at 9 and 18 months compared with baseline 2. Changes in mBMI at 9 and 18 months compared with baseline 3. Changes in R-ODS at 9 and 18 months compared with baseline 4. Percentage reduction of serum TTR level at 9 and 18 months compared with baseline 5. Overall mortality and/or overall hospitalization rate up to month 18 6. Overall mortality and/or overall hospitalization rate of the participants with cardiac involvement up to 18	Study currently recruiting	To be reported	Not licensed

Sub- stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status
Inotersen NEURO-TTR Benson et al. 2018 (NCT01737398) (29)	Phase III, multicenter, double-blind, placebo-controlled, 2:1 randomization (inotersen 300 mg s.c. vs. placebo)	Pat. (18–82 y) with neuronal manifestation of ATTRv amyloidosis n = 173, cardiac involvement in n = 108 (63%) – Inotersen n = 112 – Placebo n = 60 – 81% without premature discontinuation – 64 weeks; observation period 66 weeks	Primary: 1. Change in mNIS+7 composite score (baseline vs. week 66) 2. Change in Norfolk QoL DN Questionnaire (baseline vs. week 66) Secondary: 1. Changes in Norfolk QoL-DN Symptoms Domain score (baseline vs. week 66) 2. Changes in Norfolk QoL-DN Physical Functioning/Large-Fiber Neuropathy Domain score (baseline vs. week 66) 3. Change in mBMI (baseline vs. week 65) 4. Change in BMI (baseline vs. week 65) 5. Changes in NIS (baseline vs. week 66) 6. Changes in modified+ 7 score (baseline vs. week 66) 7. Changes in NIS + 7 (baseline vs. week 66) 8. Change in GLS (baseline vs. week 65) 9. Change in GLS in cardiomyopathy subgroup (baseline vs. 65 weeks) 10. Changes in TTR level (baseline vs. week 65) 11. Changes in RPB level (baseline vs. week 65) 12. Peak measured inotersen concentration 13. Other pharmacokinetic/pharmacodynamic parameters	– Change in mNIS+7: –19.7 points [–26.4; –13.0]; (p < 0.001) – Change in Norfolk QoL-DN score –11.7 points [–18.3; –5.1]; (p < 0.001) – Independent of disease stage, mutation type, and possible cardiomyopathy	Glomerulonephritis, thrombocytopenia (3%, 1 pat. died of cerebral hemorrhage due to grade-IV thrombocytopenia) 5 pat. died overall (4 due to underlying disease)	Licensed in Germany for pat. with grade I–II polyneuropathy in ATTRv amyloidosis; phase-III trial for pat. with ATTR cardiomyopathy started
EGCG Kristen et al. 2012 (e8)	Single-center study	Pat. with ATTR-related cardiomyopathy – n = 19 (ATTRv n = 10, 53%; ATTRwt n = 9, 47%) – EGCG for at least 12 months	Primary: Left-ventricular wall thickness	– Study drop-outs: n = 2 deaths, n = 2 discontinuation of EGCG intake, n = 1 due to heart transplantation – 2 pat. died (heart failure after 1 st month of treatment; multiorgan failure on drainage of a retro-peritoneal hematoma after a complicated disease course with several months of intensive care) – Total cholesterol 193 ± 9 (baseline) ► 173 ± 9 mg/dL (12 months) (p < 0.01); LDL 106 ± 8 ► 90 ± 8 mg/dL (at 12 months) (p < 0.01); HDL 59 ± 4 (baseline) ► 55 ± 5 mg/dL (12 months) (p < 0.01) – Decrease in septal thickness of 6.5% (p = 0.008): decrease in 12/14 pat. (86%) – Posterior wall unchanged (p = 0.03) – No increase in left ventricular mass (p = 0.084) – Significant improvement in the systolic velocity of the lateral mitral annulus (MASV) (p = 0.022) – Decrease in LV mass of 12.5% (p = 0.004) – LVEF unchanged (56.7 ± 4.7 vs. 57.2 ± 3.7%; ns.)	No SAEs	Not licensed

Sub-stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status
aus dem Siepen et al. 2015 (e9)	Single-center study	Pat. with ATTR-related cardiomyopathy (64–80 y) – n = 25 (only ATTRwt) – 600 mg EGCG for at least 12 months	Primary: Left-ventricular mass and ejection fraction (measured on cMRI)	<ul style="list-style-type: none"> – Decrease in LV mass of 6% on cMRI (196 g [100; 247] vs. 180 g [85; 237]; p = 0.03) – Decrease in total cholesterol of 8.4% (191 [118; 267] vs. 173 [106; 287] mg/dL; p = 0.006) – LVEF stable on cMRI (53% [33%; 69%] vs. 54% [28%; 71%]; p = 0.75) – Echocardiographically documented stable left ventricular wall thickness (17 [13; 21] vs. 18 [14; 25] mm; p = 0.1) – Echocardiographically documented stable MAPSE (10 [5; 23] vs. 8 [4; 13] mm; p = 0.3) 	None reported	Not licensed
EGCG Cappelli et al. 2018 (e10)	Single-center study, retrospective	Pat. with ATTR-related cardiomyopathy – EGCG 675 mg/d for at least 9 months – n = 30 (EGCG: ATTRwt n = 21, ATTRv n = 9) vs. n = 35 (control group: ATTRwt n = 30, ATTRv n = 5)	Primary: Overall mortality	– No survival advantage with EGCG ($60 \pm 15\%$ [EGCG] vs. $61 \pm 12\%$ [placebo], p = 0.276)	Study discontinuation due to diarrhea in 2 pat., no other AEs	Not licensed

Sub-stance/ study	Design	Population/ observation period	Endpoints	Effects and spread [95% confidence interval]	Adverse events	Licensing status
Doxycycline	(NCT03481972)	Cardiomyopathy in ATTRwt and ATTRv – 102 participants planned – Observation period 30 months	Primary: Efficacy of doxycycline/TUDCA Overall mortality at 18 months Secondary: Overall mortality at 18 and 30 months	Study currently recruiting	To be reported	Not licensed
	Obici et al. 2012 (e11) (NCT01171869)	Phase II, single center, open-label study (doxycycline 2 × 100 mg/TUDCA 250 mg 3 ×/d) Pat. with symptomatic neuronal or cardiac manifestation of ATTR amyloidosis (>18 y) – 20 pat. included – Observation period 12 months	Primary: Treatment response (defined as mBMI reduction <10% and NIS-LL change <2 [in PNP] or NT-proBNP increase <30% or 300 pg/mL [in isolated cardiomyopathy]) Secondary: 1. Safety and tolerance (hematological, hepatic, renal) 2. SF-36 3. Response regarding autonomic dysfunction, sensorimotor peripheral neuropathy and visceral organ involvement 4. Echocardiographic parameters 5. Doxycycline pharmacokinetics 6. Nerve conduction velocity, study discontinuation due to clinical and laboratory AEs	– 7 pat. tolerated 12 months, 10 pat. 6 months of treatment; 2 treatment discontinuations within first 2 months; 1 pat. lost during follow-ups – Therapeutic doxycycline doses documented at 6 months (7 ± 2.4 ug/mL), mean increase to 1.3 ug/mL 2 h after intake – Primary endpoint: PNP n = 6, 12 months' treatment: NIS-LL >2 in 1 pat., NIS-LL stable in 4 pat., improvement in 1 pat. with stable mBMI – Cardiomyopathy subset (n = 7), 12 months' treatment: NT-proBNP increased in 3 pat., stable in 4 pat.; echocardiographically stable in 5 pat., improved in 2 pat.; no change in NYHA class – Increasing improvement in QoL at 6 and 12 months (PCS and MCS, not statistically significant) – Kumamoto score at 6 and 12 months worse in 1 pat., better in 1 pat., and stable in 4 pat.	No SAEs; stomach ache, persistent nausea, loss of appetite	Not licensed

AEs, Adverse events; cMRI, cardiac magnetic resonance imaging; EGCG, epigallocatechin-3-gallate; GLS, global longitudinal strain; KCCQ, Kansas City Cardiomyopathy Questionnaire;

LTX, liver transplantation; LVEF, left ventricular ejection fraction; MAPSE, mitral annular plane systolic excursion; mBMI, modified body mass index; MCS, mental component scale;

mNIS + 7, modified seven-item neuropathy impairment score; NIS-LL, neuropathy impairment score in the lower limbs; NNT, number needed to treat; Norfolk QoL-DN, Norfolk quality of life-diabetic neuropathy; Norfolk QoL-DN TQOL score, Norfolk QoL-DN Total Quality of Life score; NT-pro BNP, N-terminal pro brain natriuretic peptide; NTSFnds, Summated 3 score for small nerve fiber function; pat., patient(s); PCS, physical component scale; PNP, peripheral polyneuropathy;

R-ODS, Rasch-built overall disability scale, SAEs, severe adverse events; SF-36, 36-item short form quality of life questionnaire; Summated 7 score, Summated 7 score for large nerve fiber function; y, years;