

Review

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Recent advances in the pharmacotherapy of TTR amyloidosis of the heart

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Abstract

Transthyretin amyloidosis of the heart, or transthyretin amyloid cardiomyopathy (ATTR-CM), once thought to be a rare disease, is now increasingly recognized as a common causing of restrictive cardiomyopathy, particularly in elderly patients and patients with heart failure with preserved ejection fraction. ATTR-CM is caused by an aggregation of misfolded transthyretin (TTR) protein amyloid fibrils in the myocardium. The TTR protein itself can be either wild-type (ATTRwt) or one of many pathologic variants (ATTRv). Recognition of ATTR-CM has been aided by rapid advances in technologies to diagnose the disease more accurately. Several advances in pharmacotherapeutic treatments have significantly reduced the morbidity and mortality of the disease. Treatments broadly fall into three categories: (1) TTR silencing through mRNA knockdown or silencing; (2) TTR stabilization; and (3) TTR resorption or extraction. This review article provides a survey of the pharmacokinetic and clinical data on all currently available treatments.

Keywords: Transthyretin amyloid cardiomyopathy, TTR silencing, TTR stabilization, TTR resorption, small interfering RNA, antisense oligonucleotides, carboxylic acid derivate, salicylic acid derivative

INTRODUCTION

Transthyretin amyloid cardiomyopathy (ATTR-CM) is restrictive cardiomyopathy caused by an abnormal extracellular deposition of amyloid fibrils in the myocardium. Transthyretin (TTR), also known as prealbumin, is a protein synthesized primarily by the liver and is responsible for the transportation of



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thyroxine hormone and retinol-binding protein^[1]. The full scope of its physiologic role and mechanism of degradation remain incompletely elucidated; however, the predominant hypothesis is that the protein is naturally unstable and prone to misfolding *in-vivo*. TTR protein can be either wild-type (ATTRwt) or one of many pathologic variants (ATTRv). Misfolded TTR protein molecules aggregate into amyloid fibrils and deposit in several organs of the body. However, the myocardium and autonomic nervous system appear to be most susceptible^[2]. Individual survival is most strongly correlated with the degree of myocardial deposition and the resultant severity of restrictive physiology^[3].

ATTR has a male-predominant distribution and appears to be more common than previously estimated. Myocardial ATTRwt deposition may be present in up to 10% of all elderly patients with heart failure^[4] and one-third of elderly patients with heart failure with preserved ejection fraction^[5]. Additionally, the *p.V142I*, formerly referred to as *V122I*, variant may be present in up to 3.4% of all African Americans and up to 10% of African Americans with heart failure^[6]. Median survival after initial diagnosis is 3.6 years for ATTRwt and 2.5 years for ATTRv due to the *p.V142I* mutation.

The cornerstone of treatment in ATTR-CM remains prompt diagnosis, particularly in high-risk populations. Cardiac amyloidosis is initially suspected based on characteristic findings on echocardiography and/or cardiac magnetic resonance imaging. Alternative types of cardiac amyloidosis, specifically light chain amyloidosis (AL), are then screened for with the use of serum-free light chain testing and immunofixation electrophoresis testing^[7]. Definitive diagnosis of ATTR previously required endomyocardial biopsy, but now can more readily be made using scintigraphy with bone-avid radiotracers such as ^{99m}Tc-pyrophosphate, ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid, and ^{99m}Tc-hydroxymethylene diphosphonate^[8]. Once AL amyloidosis is ruled out on laboratory testing, a diagnosis of ATTR-CM should be followed up with genetic testing to differentiate ATTRwt from ATTRv. Hereditary amyloidosis should prompt genetic counseling, screening of first-degree family members, and evaluation and treatment of neuropathy, which is more common in ATTRv. Biomarkers such as circulating retinol-binding protein 4 or misfolded ATTR oligomers may serve supplementary roles in select populations; however, further studies are required before their routine clinical use.

Symptomatic management includes diuresis with loop diuretics, blood pressure control if needed without causing orthostatic hypotension, and arrhythmia management^[9]. Patients with cardiac amyloidosis typically poorly tolerate many of the cornerstones of conventional heart failure therapy, especially beta-blockers. The role of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and angiotensin receptor-neprilysin inhibitors remains unknown.

More recently, disease-specific pharmacotherapies for ATTR-CM have emerged and become more readily available. These advances in the pharmacotherapeutic management of the disease have shown significant improvements in both patient morbidity and mortality. Pharmacotherapy can be subdivided into the following categories: (1) TTR silencing through mRNA knockdown or silencing; (2) TTR stabilization; and (3) TTR resorption or extraction [Table 1]. As these classes of medications target distinct steps in the production and degradation of the TTR molecule, combination therapy is possible, and indeed may be beneficial.

TTR SILENCING

Small interfering messenger RNA

Production of the TTR molecule, whether wild-type or variant, can be reduced by inhibiting the translation of its associated messenger RNA (mRNA). Through the activity of small interfering RNA (siRNA),

Table 1. Pharmacotherapies used in transthyretin amyloid cardiomyopathy (ATTR-CM)

Drug name	Class	Dosing	FDA approval	Key points	Notable adverse effects
TTR silencing					
Patisiran	siRNA	0.3 mg/kg IV, every 3 weeks	Yes, for hATTR polyneuropathy	Mortality benefit ^[13] , improvement in neuropathy impairment scores ^[13] , potential reduction in cardiac hospitalizations and deaths ^[14]	Vitamin A deficiency, infusion-related reactions
Revusiran	siRNA	500 mg SQ, weekly (daily during initiation)	No	Phase III trial terminated early due to increased mortality in treatment arm ^[15]	Sudden cardiac death, congestive cardiac failure
Vutrisiran	siRNA	25 mg SQ, every 12 weeks	Yes (FDA fast track), for hATTR polyneuropathy	Phase 3 trial recently completed, but data not yet presented or published ^[16]	Not reported
Inotersen	ASO	300 mg SQ, weekly (3 times weekly during initiation)	Yes, for hATTR polyneuropathy	Improvement in neuropathy impairment scores ^[18] , trend towards mortality benefit ^[18] requires weekly CBC and biweekly BMP and UA	Thrombocytopenia, glomerulonephritis, vitamin A deficiency, infusion-related reactions
AKCEA-TTR-LRx	ASO	45 mg and 90 mg SQ, every 4 weeks	No	Targeted delivery of inotersen-like compound to liver; may reduce safety concerns Phase 3 clinical trials underway (CARDIO-TTRransform and NEURO-TTRransform)	Headaches, liver enzyme abnormalities, increase in blood creatine phosphokinase, flu-like illness
TTR stabilization					
Tafamidis meglumine	Benzoxazole derivative	80 mg PO, daily (4 × 20 mg pills)	Yes	Reduction in all-cause mortality and cardiovascular hospitalization ^[20]	Allergic reactions, GI distress, headache
Tafamidis free acid	Benzoxazole derivative	61 mg PO, daily (single pill)	Yes	Lower pill burden than tafamidis meglumine ^[21] Potentially less GI distress ^[21]	Allergic reactions, GI distress, headache
Diflunisal	NSAID	250 mg PO, twice daily	Yes, off label	May have comparable mortality benefit as tafamidis ^[23] , avoid concomitant NSAID use, administer with PPI	Thrombocytopenia, renal dysfunction, fluid retention
AG-10	Novel small molecule	400 mg or 800 mg PO, twice daily	No	More robust response in ATTRm than ATTRwt ^[24] , Phase III trial underway	Atrial fibrillation, congestive heart failure, cellulitis, and dyspnea
Tolcapone	COMT inhibitor	100 mg PO, three times daily	Yes, for Parkinson's disease	<i>In vitro</i> data only currently available ^[25] , prospectively trial underway	FDA black box warning for hepatotoxicity Dyskinesia, GI distress, sleep disturbance
TTR resorption					
Miridesap with dezamizumab	Ligand and monoclonal antibody	Miridesap: 20 mg/h IV for 3 days, followed by SQ three times daily Dezamizumab: up to 1200 mg IV on days 1 and 3	No	Phase II study terminated early ^[26]	Serious rashes, others not reported
misTTR	Monoclonal antibody	0.1-30 mg/kg IV, every 28 days	No	Phase I study recently completed ^[28]	Fall, anemia, URI, back pain, GI distress, insomnia
Doxycycline with TUDCA	Tetracycline antibiotic	100 mg PO, twice daily 250 mg PO, 3 times daily	No	Phase II study showed reduction disease progression ^[29] , but high rate of serious adverse effects in follow-up study	Rash, GI distress
Others					

EGCG	Catechin	600 mg PO, daily	No	Naturally-occurring compound in green tea and turmeric, limited data in humans ^[30]	Unknown
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TTR: Transthyretin; COMT: catechol-O-methyltransferase; EGCG: epigallocatechin-3-gallate; TUDCA: tauroursodeoxycholic acid; ASO: antisense oligonucleotide.

expression of the *TTR* gene can be downregulated through an endogenous cellular mechanism. The siRNA molecules are selectively delivered to the hepatocytes where *TTR* gene expression is most avid via a lipid nanoparticle vehicle^[10].

Patisiran (ONPATTRO®, Alnylam Pharmaceuticals) binds to the 3' untranslated region of the TTR mRNA, resulting in impaired transcription of the protein and enzymatic degradation of the mRNA molecule^[11]. Because it targets a conserved region of the RNA sequence, patisiran is effective in both ATTRwt and ATTRv and can reduce circulating TTR protein levels by up to 90%^[12]. In the Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran in TTR-Mediated Polyneuropathy (APOLLO) trial, 225 patients with ATTRv polyneuropathy and New York Heart Association (NYHA) functional class I or II symptoms received patisiran vs. placebo in 2:1 randomized fashion. Patisiran was administered at a dose of 0.3 mg/kg every 3 weeks intravenously for 18 months following pre-medication with steroids, antihistamines, and acetaminophen. Treatment with patisiran showed statistically significant improvement in both morbidity and mortality compared to placebo^[13]. Patisiran-treated patients had improvements in the modified neuropathy impairment score + 7 at 18 months, the Norfolk quality of life score, 10-min walk distance, and modified body mass index. Adverse reactions consisted primarily of mild or moderate infusion-related reactions and occurred in approximately 20% of patients in the patisiran arm compared to 10% in the placebo arm.

Because concomitant cardiac involvement is seen in over half of the patients with hereditary transthyretin-mediated polyneuropathy, a subgroup analysis on a pre-specified subpopulation of patients with evidence of cardiac amyloid involvement, determined by left ventricular wall thickness ≥ 13 mm with no history of hypertension and no aortic valve disease, was conducted^[14]. At 18 months, patisiran resulted in a decrease in left ventricular wall thickness by an average of 0.9 mm, an improvement in the global longitudinal strain by 1.4%, increase in end-diastolic volume by 8.3 mL, increase in cardiac output by 0.38 L/min, and reduction in circulating NT-pro-BNP levels by 65%. A post-hoc exploratory analysis, though insufficiently powered for statistical comparison, found that the rates of both all-cause and cardiac-specific hospitalizations and deaths were lower with patisiran compared to placebo. All-cause deaths were 7 vs. 6 (4.7% vs. 7.8%), and cardiac hospitalizations and/or deaths per 100-years were 10.1 vs. 18.7 with patisiran vs. placebo. Rates of cardiac arrhythmias were also lower in the patisiran group compared to placebo; however, atrioventricular block requiring pacemaker support was seen more frequently with patisiran, in 4 vs. 0 (2.7% vs. 0%) patients.

Another siRNA molecule, revusiran (ALN-TTRSC, Alnylam Pharmaceuticals), has also been developed. It is comprised of a siRNA directed against a conserved region of the TTR mRNA that is conjugated to a triantennary N-acetylgalactosamine (*GalNAc*) ligand that delivers it directly to the liver. Recently, the Phase 3 Multicenter, Multinational, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ALN TTRSC in Patients

With TTR Mediated Familial Amyloidotic Cardiomyopathy (ENDEAVOUR) trial of revusiran was terminated early due to increased mortality in the treatment arm at a pre-specified interim analysis at 6.7 months^[15]. The majority of deaths were cardiovascular in etiology, but no clear causative mechanism was identified.

The latest generation of small interfering RNA, vutrisiran (ALN-TTRSC02, Alnylam Pharmaceuticals) is also a *GalNAc* ligand that targets the same site of the 3' untranslated region of the TTR mRNA as revusiran. However, vutrisiran potentially has fewer off-target effects through two modifications at the 5' terminus of the siRNA and a reduction in the number of 2'-fluoro-modified sugar residues, which increases the stability of the molecule and decreases the amount of 2'-fluoro nucleoside metabolites. The recently completed Phase 3 Global, Randomized, Open-label Study to Evaluate the Efficacy and Safety of ALN-TTRSC02 in Patients with Hereditary Transthyretin Amyloidosis (HELIOS-A) clinical trial investigated the efficacy of vutrisiran in the treatment of patients with hereditary ATTR polyneuropathy. A total of 164 patients were randomized in a 3:1 fashion to receive vutrisiran 25mg SQ once every 12 weeks or patisiran 0.3 mg/kg IV once every 3 weeks. For efficacy analysis, the vutrisiran arm was compared to the placebo arm of the APOLLO trial. The results of the trial will be presented on April 19, 2021 at the American Academy of Neurology Virtual Annual Meeting 2021. However, a press release from the trial's sponsor states that vutrisiran demonstrated improvements in the progression of the modified Neurologic Impairment Score + 7 (mNIS + 7), the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) score, and the timed 10-meter walk test compared to the historical control^[16]. The ongoing Phase 3, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy and Safety of Vutrisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (HELIOS-B) clinical trial is investigating the efficacy of vutrisiran in reducing a composite of all-cause mortality and recurrent cardiovascular hospitalizations in patients with hereditary ATTR amyloidosis with cardiomyopathy.

Antisense oligonucleotides

TTR protein synthesis in the hepatocytes can also be downregulated by antisense oligonucleotides (ASOs), which bind the gene's transcribed mRNA and prevent translation. ASOs are short 20 nucleotide-long chimeric RNA sequences that complementarily bind a target mRNA, leading to its rapid degradation through RNaseH and effective gene expression inhibition^[10]. ASOs have a 5'-10'-5' structure of five 2'-O-methoxyethyl modified ribonucleotides at each terminus and a central region of ten 2'-deoxynucleotide residues.

Inotersen (TEGSEDI®, Akcea Therapeutics) is a chimeric 2'-O-methoxyethyl-modified antisense oligodeoxynucleotide that binds to a 3' untranslated region of the TTR mRNA, allowing for complementary binding to the mRNA of both wild-type gene and all known hereditary variants of the gene^[17]. Inotersen reduces hepatic TTR mRNA and protein concentrations by approximately 70%, with peak reduction occurring 2-3 days after drug administration. There is gradual recovery to pre-treatment levels after 1-2 weeks and near-complete recovery after approximately 28 days. Because inotersen does not cross the blood-brain barrier, TTR production in the choroid plexus in the central nervous system remains unaffected.

The Phase 3 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 420915 in Patients With Familial Amyloid Polyneuropathy (NEURO-TTR) compared inotersen vs. placebo in patients with hereditary ATTR polyneuropathy. A total of 172 patients were randomized in a 2:1 fashion to treatment vs. placebo, 63% of the enrolled patients had cardiomyopathy, and it was more prevalent in the treatment arm than placebo at the time of trial entry. Inotersen was dosed at 300 mg subcutaneously injection three times weekly for the first week of treatment, followed by weekly injections

for a total of 64 weeks. All patients were empirically given vitamin A supplementation due to TTR's role as a transport protein for retinol. At 66 weeks, inotersen compared to placebo showed a significant decrease in the modified Neuropathy Impairment Score + 7 (mNIS + 7) score by an average of 19.7 points and a decrease in the Norfolk QOL-DN score by an average of 11.7 points. There were five deaths in the inotersen arm and zero in the placebo arm. The majority of the deaths in the inotersen arm were from the progression of underlying disease. Serious adverse reactions in the inotersen arm included glomerulonephritis (3%) and thrombocytopenia (3%), with 1 death associated with severe thrombocytopenia^[18]. Inotersen was granted Food and Drug Administration (FDA) approval for hereditary ATTR polyneuropathy with the requirement for weekly monitoring of platelet counts and biweekly monitoring of renal function and urinary protein.

A small open-label study of inotersen in 15 patients with either hereditary or wild-type ATTR cardiomyopathy showed disease stabilization after 12 months of treatment. Inotersen use resulted in improved 6-min walk distance by an average of 29.2 meters, reduction in left ventricular mass by an average of 27 grams, change in global longitudinal strain by an average of -1.6, and reduction in circulating BNP levels by an average of 61 pg/mL^[19].

Similar to siRNA therapy, ASO therapy showed cardiovascular benefit after an average of 15-18 months of use, later than the 9 months to see differences in neurologic outcomes in patients with hereditary TTR amyloidosis.

A next-generation of ASO, AKCEA-TTR-L_{RX} (ION-682884, Ionis Pharmaceuticals) is now being studied, targeting the same sequence as inotersen but also conjugated to a triantennary N-acetylgalactosamine (*GalNAc*) ligand, to allow increased delivery to the liver and decrease the total dose required with hopes of alleviating some of the safety concerns with inotersen. The Phase 3 Global, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ION-682884 in Patients with Transthyretin-Mediated Amyloid Cardiomyopathy (CARDIO-TTRansform) and Phase 3 Global, Open-Label, Randomized Study to Evaluate the Efficacy and Safety of ION-682884 in Patients with Hereditary Transthyretin-Mediated Amyloid Polyneuropathy (NEURO-TTRansform) clinical trials are currently underway.

Both siRNAs and ASOs can be effectively used to silence *TTR* gene expression and protein production. They have therapeutic potential in the treatment of TTR cardiomyopathy when present with neuropathy; the role of TTR silencing in the treatment of isolated cardiomyopathy is still under investigation. Several randomized clinical trials, including APOLLO-B, HELIOS-B, CARDIO-TTRansform, and NEURO-TTRansform, are ongoing^[7].

TTR STABILIZATION

Tafamidis

The most well-known and widely available treatment for transthyretin amyloid cardiomyopathy is tafamidis (Vyndaqel®, Pfizer Inc), a TTR protein stabilizer. Tafamidis is a benzoxazole derivative that binds TTR protein molecules at the thyroxine-binding site with high affinity. Hydrogen bonding stabilizes the TTR tetramer and prevents the creation of monomers that would pathologically aggregate into amyloid fibrils subsequently. Tafamidis is a carboxylic acid derivative; however, it does not have anti-inflammatory properties.

The Multicenter, International, Phase 3, Double-Blind, Placebo-Controlled, Randomized Study to Evaluate the Efficacy, Safety, and Tolerability of Daily Oral Dosing of Tafadmis Meglumine PF-06291826 20 MG or

80 MG in Comparison to Placebo in Subjects Diagnosed with Transthyretin Cardiomyopathy (ATTR-ACT) clinical trial compared tafamidis meglumine vs. placebo in patients with either wild-type or hereditary ATTR cardiomyopathy. A total of 441 patients were randomized in a 2:1:2 fashion to treatment with tafamidis at 80 mg daily vs. tafamidis at 20 mg daily vs. placebo. 76% of the enrolled patients had wild-type TTR amyloidosis, and the patients across all study arms were predominantly males. Tafamidis was dosed at either 80 mg or 20 mg once daily for a total follow-up of 30 months. Outcomes analysis was done using the Finkelstein-Schoenfeld method, a hierarchical rank-sum analysis. Firstly, tafamidis resulted in an average decrease in all-cause mortality by 30%, with an average overall mortality of 29.5% vs. 42.9% at 30 months in the tafamidis vs. placebo. This equated to a 13.4% absolute difference in mortality, and a number of 7.5 patients needed to treat to prevent 1 death after 30 months of treatment. Secondly, the annual cardiovascular-related hospitalization rate was 0.48 vs. 0.70 in the tafamidis vs. placebo arms, a 32% relative risk reduction in cardiovascular hospitalizations^[20]. Secondary outcomes analyses showed tafamidis resulted in improved 6-min walk distance by an average of 75.7 meters, reduction in the decline in KCCQ-OS score by an average of 13.7 points, a smaller increase in NT-pro-BNP levels by an average of 735 pg/mL at 12 months, and 2181 pg/mL at 30 months, and a smaller decrease in left ventricular stroke volume by an average of 6.3 mL at 30 months^[20]. Kaplan-Meier survival analysis revealed that tafamidis showed mortality benefit after an average of 18 months of treatment. Improvements in 6-min walk distance and quality-of-life metrics like the KCCQ-OS questionnaire were noted in the tafamidis arm at 6 months.

There were no serious adverse effects that were more common in the tafamidis arms than placebo. Interestingly, the pre-specified subgroup of patients with NYHA functional class III symptoms prior to enrollment showed an increased rate of cardiovascular-related hospitalizations in the tafamidis vs. control arm.

Tafamidis meglumine was granted FDA approval for ATTR cardiomyopathy of either wild-type or hereditary etiology. Tafamidis has rapidly become the most widely-used pharmacotherapy for ATTR cardiomyopathy; however, associated costs remain a significant barrier to broader access. Dosing initially was 80 mg daily (4 × 20mg pills) in cardiomyopathy. Tafamidis free acid (Vyndamax®, Pfizer Inc) 61 mg daily is now available and comes with the advantage of a lower pill burden compared to tafamidis meglumine^[21].

Diflunisal

Diflunisal (Dolobid®, Merk & Co) is a decades-old generic nonsteroidal anti-inflammatory molecule, a salicylic acid derivative, which has more recently been found to be complex with the TTR protein's two thyroxine binding sites. The resultant protein complex stabilizes the TTR tetramer form and inhibits protein misfolding that would otherwise result in degradation and the production of TTR monomers. Initial research on diflunisal employed the National Institutes of Health mission to repurpose old drugs and focused on hereditary TTR amyloid polyneuropathy. It was subsequently followed by single-center open-label and retrospective studies on the use of diflunisal in TTR amyloid cardiomyopathy^[22,23]. Approximately 57%-90% of patients managed with diflunisal had ATTRwt cardiomyopathy, 79%-82% had either NYHA functional Class I or II symptoms, 83%-87% of the patients were of the white race, and 87%-88% were males. Diflunisal was dosed at 250 mg orally twice daily, and the median duration of therapy ranged from 15-23 months. In Kaplan-Meier analysis, diflunisal was associated with an improvement in the time to death or heart transplantation, with a median time of 5.4 years compared to 2.2 years for patients with ATTR-CM not on treatment^[23]. This was comparable to outcomes with tafamidis. Based on this limited data, it is hypothesized that the survival benefit with diflunisal may be comparable with tafamidis. Diflunisal appears to be generally well-tolerated, with low rates of thrombocytopenia and renal dysfunction. Gastrointestinal adverse effects are less common but in the setting of routine concomitant use of histamine blockers or

protein pump inhibitors for prophylaxis. Fluid retention leading to heart failure exacerbation and clinically significant bleeding were also uncommon. These findings may have been related to the use of lower diflunisal dosage (250 mg BID) for the treatment of transthyretin amyloidosis than that routinely used for the treatment of inflammatory conditions. Despite a lack of robust clinical data to guide its use, diflunisal remains an important pharmacotherapeutic tool in the management of TTR amyloid cardiomyopathy because of its favorable safety profile and low costs. However, this agent is not FDA approved for use in patients with TTR amyloidosis.

Investigational compounds

AG-10 (Acoramidis, BridgeBio LLC, formerly Eidos Therapeutics) is an investigational compound that has the potential to stabilize TTR tetramers and prevent their degradation into amyloidogenic monomer fibrils. The compound was developed based on the discovery of the T119M is a stabilizing mutation of TTR. This reduces TTR degradation by 33-fold compared to the wild-type protein. AG-10, like the *T119M* TTR variant protein, promotes the formation of hydrogen bonds with serine residues at position 117 and promotes TTR stabilization. A recent phase II clinical trial of AG-10 with 49 patients showed that it was effective and generally well-tolerated in patients with ATTR-CM and NYHA functional class II or III symptoms and baseline high circulating NT-pro-BNP levels^[24]. AG-10 led to an average increase in circulating TTR levels of 36% with 400 mg dosing and 50% with 800 mg dosing. Patients with variant or hereditary TTR (ATTRv-CM) had a more robust response than those with wild-type ATTR cardiomyopathy. Compared to placebo, AG-10 had a slightly higher occurrence of atrial fibrillation, constipation, diarrhea, and muscle spasms. Serious adverse effects with AG-10 included atrial fibrillation, congestive heart failure, cellulitis, and dyspnea requiring hospitalization. A phase III randomized clinical trial of AG-10, ATTRIBUTE-CM, is currently ongoing.

Finally, tolcapone (TASMAR®, Valeant Pharmaceuticals) is an orally active catechol-O-methyltransferase (COMT) inhibitor that binds to the TTR thyroxine-binding pocket with high affinity. The drug is already available for the treatment of Parkinson's disease as an adjunct to carbidopa/levodopa. Tolcapone increases the concentration of dopamine in the central nervous system by further reducing its degradation. It carries an FDA black box warning for hepatotoxicity, and was previously removed from the market for safety concerns before being re-instated. It is now being studied in ATTR. *In vitro*, it has a higher affinity for the binding pocket than even tafamidis and was able to reduce degradation of highly unstable TTR variant tetramers by 40%-50% and degradation of wild-type TTR tetramers by nearly 80%. A prospective clinical trial is currently underway^[25].

TTR RESORPTION

Antibody-mediated removal

Serum amyloid protein (SAP) is a non-fibrillar normal plasma-circulating protein that is also bound with pathogenic TTR amyloid fibrils. Targeted removal of plasma SAP with the ligand miridesap (GSK2315698, GlaxoSmithKline, and Pentraxin Therapeutics), previously known as CPHPC, leaves behind fibrillin-bound SAP. This can then be targeted and bound by a therapeutic IgG1 anti-SAP antibody, dezamizumab (GSK2398852, GlaxoSmithKline and Pentraxin Therapeutics), which activates a complement-based cascade that could mark TTR amyloid fibrils for destruction by macrophage-derived multinucleated giant cells. A phase I clinical trial of this antibody-mediated approach to TTR degradation and resorption initially showed promise; however, further study was later paused due to futility and toxicity^[26]. Some data suggest that repeat doses of dezamizumab may show clinical benefit^[27], though more investigation is needed.

MisTTR (PRX004, Prothena Corporation) is a monoclonal antibody that binds residues 89-97 of the TTR tetramer, resulting in inhibition of fibrillogenesis of misfolded TTR monomers. The misTTR antibody binds to the F-strand of the protein at the dimer interface of TTR, which is inaccessible in the native tetramer^[28]. Without amyloid fibrillin formation, the misfolded transthyretin monomers are targeted for degradation by the immune system. In preclinical studies, the antibody was able to inhibit amyloid fibril formation and promote clearance of insoluble amyloid fibrils through antibody-mediated phagocytosis. A Phase I clinical study was completed recently and showed improvement in neuropathy progression and improvement in left ventricular systolic function. Publication of the data is still pending.

Doxycycline and tauroursodeoxycholic acid

It had been hypothesized that TTR tetramers could be disrupted, and the resultant amyloid fibrils are cleared by macrophages and giant cells in the innate immune system, by doxycycline plus tauroursodeoxycholic acid (TUDCA)^[29]. Tetracycline antibiotics, such as doxycycline, had been shown to disrupt TTR fibrillin structure. TUDCA was then shown to have a synergistic effect in disrupting the fibrillin structure, and also in marking ATTR molecules for degradation. An initial phase II clinical trial, where patients were given doxycycline 100 mg twice daily and TUDCA 250 mg three times daily for a total of 12 months, showed promise in halting disease progression. However, a subsequent study showed conflicting efficacy results, and raised concerns about high rates of serious adverse effects^[29].

Naturally-derived compounds

Naturally-occurring compounds in green tea have been purported to decrease amyloid fibril monomer formation. It has been hypothesized that epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea, may inhibit amyloid fibril formation and reduce the incidence of clinical amyloidosis. This was further supported by a study in mice that showed a decrease in left ventricular mass following daily administration of EGCG to transgenic mice carrying the human Val30Met TTR variant. It was followed by a series of small prospective cohort studies in humans examining the effect of voluntary daily green tea consumption on the development of wild-type TTR amyloid cardiomyopathy. Daily consumption of 1200 mg of green tea extract containing 600 mg EGCG for a total of 12 months may be associated with a decrease in left ventricular mass by 6%-13% on cardiac MRI; however, the results do not appear to be consistently demonstrated^[30]. A single-center retrospective study examining patients treated for a minimum of 9 months with EGCG did not demonstrate a survival improvement compared to guideline-directed medical therapy alone^[31].

CONCLUSION

The pharmacotherapeutic armamentarium in the treatment of transthyretin amyloid cardiomyopathy (ATTR-CM) has expanded greatly in the last several years, as has our understanding of the prevalence of the disease. It is now commonly accepted that ATTR-CM is not a so-called orphan disease but may, in fact, afflict up to 10%-30% of all elderly patients with heart failure. Recognition of the disease has been aided by rapid advances in technologies to diagnose ATTR-CM more accurately. The treating cardiologist may also be faced with confusion in choosing an optimal pharmacotherapy regimen for treatment out of several options in the coming years. While newer agents are being developed, attention should also be given to a better understanding of the disease process, optimal screening strategies, developing treatment regimens that effectively reverse the disease process, and assessing the efficacy of treatment. Consensus is needed on identifying patient populations that warrant screening for ATTR-CM, and the imaging modalities and biomarkers to be used for efficient screening. Consensus definitions on assessing treatment response and disease progression are also needed. Finally, approaches to tailoring specific disease-modifying agents to various stages of disease severity, and consideration for combination therapy, are still being developed. It remains an exciting time for the diagnosis and treatment of ATTR-CM because of the expectation of

significant advances on the horizon.

DECLARATIONS

Authors' contributions

Contributed to the writing of this review paper: Shah RJ, Pan S, Lanier GM, Mellela L, Aronow WS, Jain D

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