

CONTEMPORARY REVIEW

Recent Advances and Current Dilemmas in the Diagnosis and Management of Transthyretin Cardiac Amyloidosis

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ABSTRACT: Cardiac amyloidosis (CA) is an increasingly recognized cause of heart failure, arrhythmias, and sudden cardiac death. While CA was previously rapidly fatal, recent advances in diagnosis and treatment have significantly improved outcomes. Advances in cardiac imaging and biomarkers have critically improved the accuracy and efficiency with which CA is diagnosed, even allowing for the noninvasive diagnosis of transthyretin CA. Cardiac magnetic resonance imaging, technetium nuclear imaging, echocardiography, and blood-based biomarkers have established important and complementary roles in the management and advancement of care. At the same time, the development of novel targeted amyloid therapies has allowed patients with CA to live longer and potentially achieve better quality of life. Still, despite this significant progress, there remain critical ongoing questions in the field. Accordingly, within this review we will highlight recent advances in cardiac imaging and therapeutics for CA, while focusing on key opportunities for further optimization of care and outcomes among this growing population. Specifically, we will discuss ongoing debates in the diagnosis of CA, including the interpretation of indeterminate cardiac imaging findings, the best technique to screen asymptomatic transthyretin amyloidosis gene mutation carriers for cardiac involvement, and the ideal method for monitoring response to CA treatment. We will additionally focus on recent advances in treatment for transthyretin amyloidosis-CA, including a discussion of available agents as well as highlighting ongoing clinical trials. Together, these data will allow clinicians to emerge with a greater understanding of the present and future of diagnosis, management, and potentially enhanced outcomes in this rapidly advancing field.

Key Words: cardiac amyloidosis ■ cardiac imaging ■ cardio-oncology ■ targeted therapies ■ transthyretin

Amyloidosis is a pathological disease process in which abnormally folded proteins deposit within various tissues, leading to marked interstitial expansion, end organ dysfunction, and damage. When it involves the heart, amyloidosis associates with particularly high morbidity and mortality.¹⁻³ The 2 most common causes of cardiac amyloidosis (CA) are transthyretin and immunoglobulin light chain proteins, which combined account for 95% of cases of CA. TTR amyloidosis (ATTR) can occur either spontaneously (ATTR wild-type) or because of a pathologic germline mutation (ATTR variant). While previously

thought to be rare, the presence of CA is increasingly recognized to be more common, with an estimated prevalence of 13% to 16% among patients presenting with heart failure with preserved ejection fraction and severe aortic stenosis.⁴⁻⁶ Recently, the development of several drugs, which stabilize or prevent the production of the TTR protein, have dramatically improved the outcomes of patients with ATTR.¹⁻³ With effective therapies now available for ATTR, it is becoming imperative to make an early diagnosis of ATTR cardiomyopathy (ATTR-CM) in order to streamline effective therapies. Significant advances in both

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Nonstandard Abbreviations and Acronyms

AL-CM	immunoglobulin light chain cardiomyopathy
ATTR	transthyretin amyloidosis
ATTR-CM	transthyretin cardiomyopathy
ATTRv	variant transthyretin amyloidosis
CA	cardiac amyloid
ECV	extracellular volume
KCCQ	Kansas City Cardiomyopathy Questionnaire
LGE	late gadolinium enhancement
Tc-DPD	technetium 3,3-diphosphono-1,2-propanodicarboxylic acid
Tc-PyP	technetium pyrophosphate

cardiac imaging and blood-based biomarkers in the field of CA have helped make this possible.

Yet, despite the recent strides there remain several key unanswered questions in the rapidly evolving field of ATTR-CM. These include:

1. The evaluation of ATTR gene mutation carriers with early (preclinical) forms of CA,
2. The interpretation of indeterminate diagnostic findings in the presence of clinically manifested disease,
3. The efficacy of applying novel therapies approved for noncardiac ATTR for the control of cardiac symptoms and pathology,
4. The optimal methods for screening asymptomatic variant transthyretin amyloidosis (ATTRv) carriers, and
5. The potential (bio)markers for monitoring response to therapy in ATTR-CM.

Accordingly, within this review we will highlight recent advances in cardiac imaging and therapeutics for CA, while focusing on key opportunities for further optimization of care and outcomes among this growing population. We will also highlight ongoing clinical trials and areas of active research within this emerging field.

CURRENT DIAGNOSTIC AND TREATMENT STRATEGIES

Diagnosis of ATTR-CM

Currently, the screening and diagnosis of CA requires a comprehensive approach with biomarkers, multimodality imaging, and invasive tissue biopsy often playing complementary roles (Table 1). Echocardiography is frequently used as an initial

screening test in patients suspected to have CA. Echocardiography typically reveals increased left and right ventricular wall thickness, bi-atrial enlargement, valvular thickening, speckled appearance of the myocardium, and/or reduced mitral annular tissue velocities (Figure 1A).^{6,7} Two-dimensional speckle tracking may reveal impairment in global longitudinal strain with or without relative apical sparing, a finding that is somewhat specific for CA.⁸ This classical strain pattern appears to be predominantly related to regional variability in amyloid mass rather than concentration when correlated with advanced imaging.⁹ Because of its tissue characterization capabilities, cardiac magnetic resonance (CMR) is particularly well suited to assess CA. Late gadolinium enhancement imaging (LGE) and extracellular volume (ECV) mapping allow for the visual and quantitative assessment of myocardial amyloid infiltration (Figure 1B).¹⁰ In CA, LGE typically begins in a subendocardial pattern and progresses to transmural involvement in the later stages of disease.¹¹ The use of pre- and postgadolinium T1 mapping allows for the quantification of ECV—the percentage of myocardium composed of the extracellular space. ECV is markedly elevated in CA and correlates with histologic amyloid burden.^{12,13}

When CA is suspected on the basis of clinical or imaging findings, patients should undergo evaluation for a monoclonal gammopathy to screen for immunoglobulin light chain cardiac amyloidosis (AL-CM). If there is no monoclonal gammopathy detected, cardiac scintigraphy with a bone-avid radiotracer, either technetium pyrophosphate in the United States, or 3,3-diphosphono-1,2-propanodicarboxylic acid in Europe, or ^{99m}Tc-hydroxymethylene diphosphate, can confirm the diagnosis of ATTR-CM. If the technetium pyrophosphate or technetium 3,3-diphosphono-1,2-propanodicarboxylic acid scan is equivocal or there is a monoclonal gammopathy, an invasive biopsy is required (Figure 1C).¹⁴ A diagnosis of CA can be made on invasive biopsy when amyloid fibrils are present on Congo Red staining.¹⁵ Amyloid subtype is then determined invasively with either laser dissection with mass spectroscopy or immunohistochemistry, with the former method being the criterion standard.¹⁶ If ATTR is detected, genetic testing is required to evaluate for the presence of a pathologic TTR gene mutation. Recently, it has been demonstrated that a reliable diagnosis of ATTR-CM can also be made noninvasively with 99% specificity when grade ≥ 2 uptake on Tc-PyP/DPD imaging is present in the absence of a serum or urine monoclonal light chain protein (Figure 1D).¹⁴ The widespread adoption of Tc-PyP/DPD has greatly increased our ability to diagnose ATTR-CM and has consequently improved patient access to potentially life-prolonging ATTR therapies. While ATTR-CM may be diagnosed

Table 1. Practical Considerations in the Diagnosis and Management of Cardiac Amyloidosis

Modality	Screening	Diagnosis/ Subtyping	Staging	Hallmark Findings	Advantages	Disadvantages
Electrocardiography	+	–	+	<ul style="list-style-type: none"> • Low voltage • Pseudoinfarct pattern • Lack of precordial left ventricular hypertrophy, disproportionate to mass 	<ul style="list-style-type: none"> • Cheap and easily obtained 	<ul style="list-style-type: none"> • Lacks sensitivity
Echocardiography	+	–	+	<ul style="list-style-type: none"> • Increased left and right ventricular wall thickness. • Bi-atrial enlargement • Valvular thickening • Impaired longitudinal strain with apical sparing 	<ul style="list-style-type: none"> • Widely available 	<ul style="list-style-type: none"> • Lacks sensitivity • Cannot determine amyloid subtype • Cannot reliably differentiate amyloid from other hypertrophic phenocopies
Cardiac magnetic resonance	+	–	+	<ul style="list-style-type: none"> • Diffuse subendocardial or transmural LGE • Markedly elevated extracellular volume (ECV) >40% • Concentric left ventricular hypertrophy • Abnormal longitudinal strain even when EF preserved • Bi-atrial enlargement • Valvular thickening 	<ul style="list-style-type: none"> • Can differentiate amyloid from other hypertrophic phenocopies • ECV correlates with amyloid burden • Diagnostic versatility enables correct diagnosis when amyloidosis not present 	<ul style="list-style-type: none"> • Cannot differentiate amyloid subtype • Need for gadolinium for LGE/ECV
Serum/urine light chain analysis	–	+	+	<ul style="list-style-type: none"> • Presence of urine or plasma monoclonal protein • K/λ ratio 	<ul style="list-style-type: none"> • Inexpensive and widely available • Prognostic in AL-CM 	<ul style="list-style-type: none"> • No role in ATTR cardiomyopathy
Tc-PyP/DPD	+	+	–	<ul style="list-style-type: none"> • Avid visual myocardial uptake equal to or greater than rib uptake • Increased heart to contralateral lung (H/CL) ratio 	<ul style="list-style-type: none"> • Highly sensitive • Able to noninvasively diagnose ATTR-CM (in absence of light chain) 	<ul style="list-style-type: none"> • Radiation exposure • Positive in up to 20% of cases of AL-CM
Cardiac biomarkers (troponin, NT-proBNP, etc)	+/-	–	+	<ul style="list-style-type: none"> • Elevation in NT-proBNP and troponin out of proportion with volume status 	<ul style="list-style-type: none"> • Cheap and easily obtained • Useful for staging 	<ul style="list-style-type: none"> • Lacks sensitivity
Tissue biopsy	–	+	–	<ul style="list-style-type: none"> • Apple green birefringence on Congo Red stain • Presence of amyloid fibrils by mass spectroscopy or immunohistochemistry 	<ul style="list-style-type: none"> • Affected organ biopsy highly sensitive and specific (criterion standard) • Able to determine amyloid subtype (regardless of light chain presence) 	<ul style="list-style-type: none"> • Invasive • Fat pad biopsy may have lower diagnostic yield

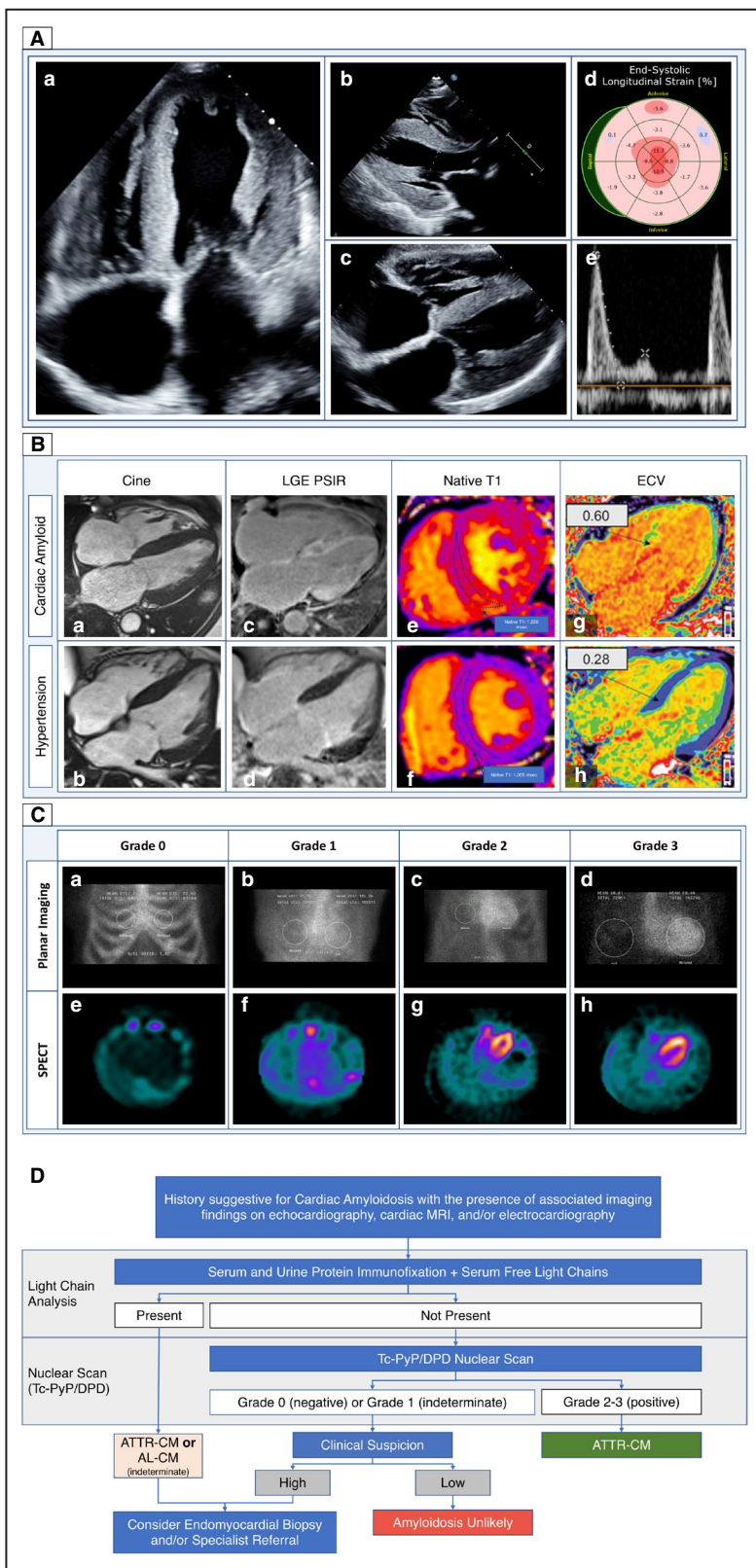
AL-CM, immunoglobulin light chain amyloidosis; ATTR, transthyretin amyloidosis; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; ECV, extracellular volume; EF, ejection fraction; LGE, late gadolinium enhancement; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PyP, pyrophosphate; and Tc, technetium.

by either nuclear scan or invasive biopsy, a diagnosis of AL-CM requires tissue biopsy.^{14,15,17} A comprehensive list of currently available diagnostics in CA is summarized in Table 1. Since prevalence of amyloidosis seldom exceeds 15% in various populations, a “Bayesian” approach might suggest that CMR is an appropriate first-line test given its diagnostic versatility to identify relevant cardiac disease when amyloidosis is not present, and ability of ECV by CMR to quantify disease burden. Bone scintigraphy scanning does not appear well suited for tracking quantitative measures of amyloid burden serially over

time; however, this is an area of growing interest.^{18–20} Further research into optimal diagnostic pathways is needed.

Transthyretin Amyloidosis Treatment

In 2018, 3 randomized controlled clinical trials transformed the landscape of ATTR treatment.^{1–3} On the basis of these compelling data, the US Food and Drug Administration approved tafamidis for the treatment of ATTR-CM and both patisiran and inotersen for hereditary ATTR polyneuropathy within the year.²¹



Patisiran—currently approved to treat hereditary transthyretin polyneuropathy—is currently under investigation for ATTR-CM. These drugs act at multiple sites along the pathway of amyloid fibrillogenesis (Figure 2).

Before these therapies, treatments available for transthyretin amyloidosis were primarily limited to off-label medications, supportive care for symptoms, and/or therapies related to specific organ dysfunction.¹⁴

Figure 1. Cardiac imaging features of cardiac amyloidosis.

A, Echocardiographic features in cardiac amyloidosis. **a** and **b**, Classic 2-dimensional images demonstrating increased left ventricular wall thickness with biatrial enlargement. **c**, Increased thickness of the right ventricle and interatrial septum. **d**, Globally impaired longitudinal strain with relative apical sparing (“cherry on top” pattern). **e**, Marked impairment in diastolic function with diminutive A-wave suggestive of atrial contractile dysfunction. **B**, CMR in CA: CMR in a patient with CA (top panel) and HHD (bottom panel). **a** and **b**, Cine images showing increased biventricular wall thickness with biatrial enlargement in both patients. With **(c** and **d)** LGE imaging demonstrating diffuse, transmural fibrosis in CA as compared with minimal replacement fibrosis in HHD. As well as **(e** through **H)** T1 mapping before **(e** and **f)** gadolinium contrast administration and ECV mapping **(g** and **h)** demonstrate markedly elevated precontrast T1 with very high ECV in CA as compared with HHD (CA indicates cardiac amyloidosis; CMR, cardiac magnetic resonance; ECV, extracellular volume; HHD, hypertensive heart disease; LGE, late gadolinium enhancement). Reprinted from *Myocardial Edema and Prognosis in Amyloidosis*, 71(25):2919–2931, Kotecha et al, ©2018 with permission from Elsevier. **C**, Technetium pyrophosphate (Tc-PyP) in CA: planar **(a** through **d)** and SPECT **(e** through **h)** imaging in Tc-PyP demonstrating examples of Grades 0, 1, 2, and 3 uptake. In grade 0 uptake, there is no cardiac uptake by either planar or SPECT imaging. In grade 1 uptake, there is slight cardiac uptake on planar and SPECT imaging that is exceeded by bone uptake. In grade 2, cardiac uptake exceeds bony uptake. In grade 3 uptake, there is diffuse cardiac uptake on planar and SPECT imaging with diminished bone uptake (SPECT indicates single photon emission computed tomography). **D**, Proposed diagnostic algorithm for suspected cardiac amyloidosis: this figure depicts the proposed algorithm for diagnosis of cardiac amyloidosis in patients suspected to have cardiac amyloidosis on the basis of suggestive clinical features and/or cardiac imaging. AL-CM indicates immunoglobulin light chain cardiac amyloidosis; ATTR-CM, transthyretin amyloidosis; and TcPyP/DPD, technetium pyrophosphate/technetium 3,3-diphosphono-1,2-propanodicarboxylic acid. Reprinted from *ASNC/AHA/ASE/EANM/HFSA/ISA/SCMR/SNMMI Expert Consensus Recommendations for Multimodality Imaging in Cardiac Amyloidosis: Part 1 of 2—Evidence Base and Standardized Methods of Imaging*, 25(11):e1–e39, Dorbala et al, ©2019, with permission from Elsevier.

However, each novel ATTR amyloidosis therapy is delivered differently with unique requirements affecting access and ease of use.²¹

Transthyretin Stabilizing Molecule Therapies

Tafamidis—a benzoxazole derivative—is an analogue of diflunisal that lacks nonsteroidal anti-inflammatory drug activity. It acts by binding the thyroxine binding site of transthyretin, stabilizing the tetramer and preventing the dissociation into monomers, which can subsequently misfold into amyloid fibrils (Figure 2). Tafamidis was inspired by the discovery of a stabilizing mutation in transthyretin, Thr119Met, which protects Val30Met mutation carriers from developing amyloidosis.²² Tafamidis is taken as a daily oral medication. The ATTR-ACT (The Transthyretin Amyloidosis Cardiomyopathy Clinical Trial) enrolled 441 patients with ATTR-CM (≈75% wild-type), randomizing 264 patients to tafamidis and 177 patients to placebo.¹ Patients on tafamidis had significantly lower all-cause mortality, lower rate of cardiovascular-related hospitalizations, and reduced decline in functional capacity/quality of life compared with placebo-treated patients. Improvements in biomarkers were noted starting at 9 months. After 18 months, survival benefit was observed. No significant adverse events were observed.¹ In our clinical experience, some patients prefer to take the tafamidis at night because of perceptions of abdominal discomfort or lethargy associated with morning dosing. No laboratory monitoring is required.

Investigational Transthyretin-Stabilizing Molecule Therapies

Other drugs that stabilize the transthyretin tetramer similar to tafamidis are also currently being tested. A

large, phase 3 trial is currently under way to assess the effects of orally administered acoramidis (AG-10) in preventing major adverse cardiovascular events in ATTR-CM (Table 2).^{22–33} In the phase 2 clinical trial, acoramidis was well tolerated and successfully reduced serum transthyretin levels.³⁴

Inhibitory Oligonucleotide Therapies: Patisiran and Inotersen

Patisiran and inotersen are both oligo-nucleotide-based therapeutic agents that target the 3′ untranslated region of the transthyretin mRNA.³⁵ Patisiran originally circulates as a double-stranded RNA molecule, subsequently dissociating into single-stranded molecules in the cytoplasm. The sense strand then functions as a small interfering RNA, binding the transthyretin mRNA to form an RNA-induced silencing complex, which is subsequently cleaved, inhibiting synthesis of the transthyretin protein (Figure 2). These agents result in decreased levels of both normal and mutant transthyretin proteins. Inotersen is an antisense oligonucleotide that binds to the target mRNA in the nucleus. This complex is then degraded by ribonuclease H (Figure 2).

Patisiran is an intravenous medication delivered every 3 weeks. To reduce the risk of an infusion reaction, patients are premedicated with a corticosteroid, acetaminophen, and antihistamines (H1 and H2 blockers). The effects of patisiran in hereditary amyloid neuropathy were studied in the APOLLO (The Study of an Investigational Drug, Patisiran [ALN-TTR02], for the Treatment of Transthyretin [TTR]-Mediated Amyloidosis) trial, which randomized 148 patients to patisiran, and 77 patients to placebo.² Patisiran-treated patients had significant improvements in both neuropathy scores and gait speed compared with baseline, while the control arm progressively worsened in both respects.² In

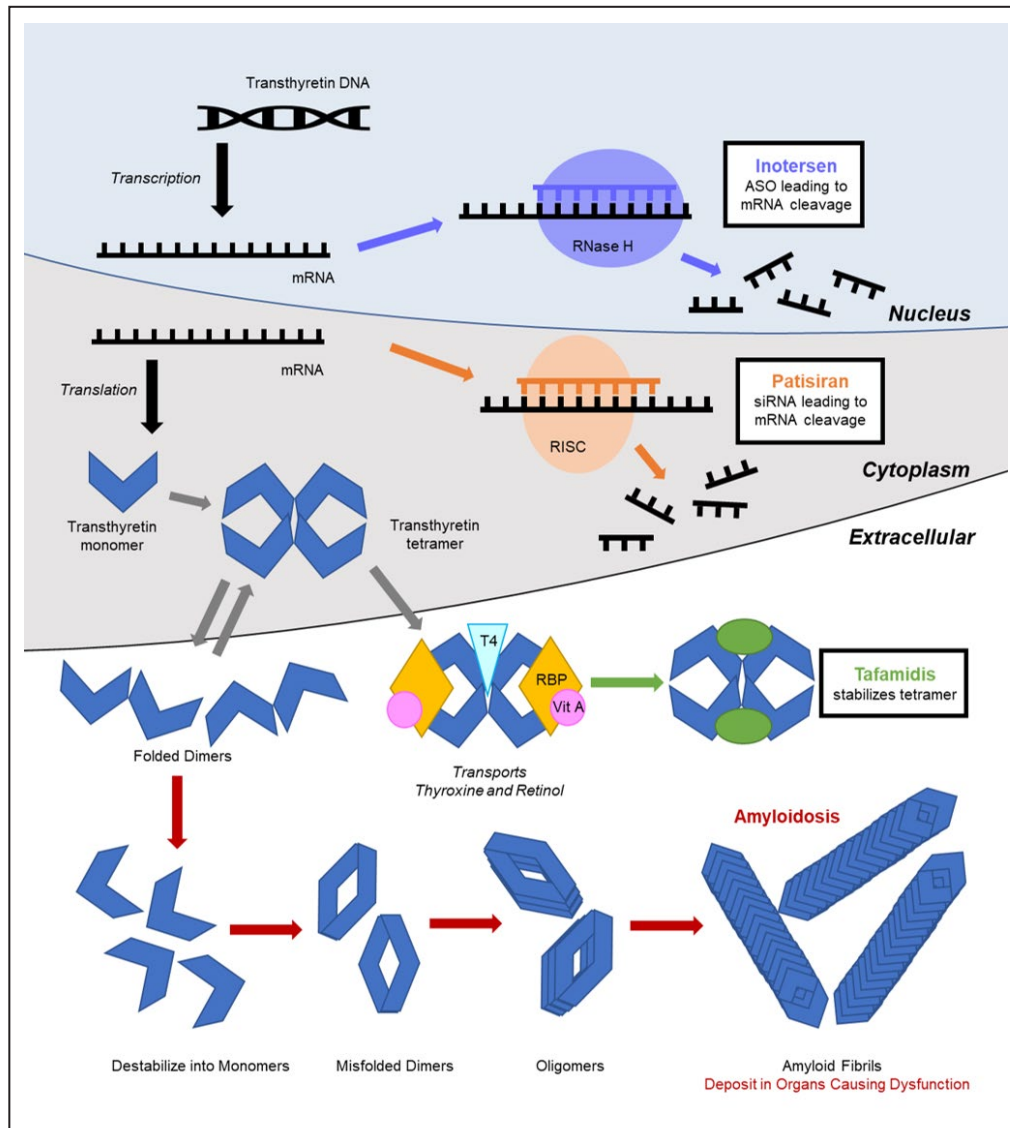


Figure 2. Basic mechanisms of amyloid fibril deposition, and targets of novel therapeutic interventions.

a subgroup analysis of patients with ATTR-CM, patisiran use was associated with reductions in left ventricular wall thickness, NT-proBNP (N-terminal B-type natriuretic peptide), and even a composite end point of cardiovascular hospitalization or mortality.³⁶

The most common adverse event in this study was an infusion reaction, which occurred in 19% of patients receiving patisiran as compared with 9% in the placebo group.² Therefore, after receiving the drug, patients must be monitored for symptoms of infusion reactions, which can include flushing, back pain, nausea, abdominal pain, shortness of breath, or headache. While some insurances cover home infusions, most patients must travel to an infusion center to receive the medication, limiting its widespread availability. Additionally, transthyretin aids in vitamin K transport via a complex with vitamin A. Therefore, all patients treated with

patisiran should be prescribed vitamin A supplementation to avoid deficiencies, which could result in ocular toxicity. No laboratory monitoring is required with this medication.

Inotersen is administered as a weekly subcutaneous injection. After observed administration by a healthcare professional for the first dose, patients can self-administer the injection at home. Inotersen was also studied in the NEURO-TTR (Efficacy and Safety of Inotersen in Familial Amyloid Polyneuropathy) trial, which randomized 172 patients with hereditary transthyretin polyneuropathy to inotersen versus placebo.³ Inotersen use was associated with greater stabilization in neuropathy scores as compared with the placebo. In the inotersen group, 3% of patients developed glomerulonephritis and 3% developed thrombocytopenia. Because of adverse events in

Table 2. Summary of Ongoing Clinical Trials and Investigational Drugs in Familial Amyloid Polyneuropathy and Transthyretin Cardiac Amyloidosis

Mechanism of Action	Drug (Route of Administration)	FDA Approval		Strength of Supportive Evidence	Active Clinical Trials
		Cardiomyopathy	Neuropathy		
siRNA	Patisiran (IV)	...	✓	Large RCT	<ul style="list-style-type: none"> • APOLLO-B: A Study to Evaluate Patisiran in Participants With Transthyretin Amyloidosis With Cardiomyopathy (ATTR Amyloidosis With Cardiomyopathy)²³ • Patisiran in Patients With Hereditary Transthyretin-mediated Amyloidosis (hATTR Amyloidosis) Disease Progression Post-Liver Transplant²⁴
	Vutrisiran (SQ)	Ongoing RCT	<ul style="list-style-type: none"> • HELIOS-A: A Study of Vutrisiran (ALN-TTRSC02) in Patients With Hereditary Transthyretin Amyloidosis (hATTR Amyloidosis) polyneuropathy²⁵ • HELIOS-B: A Study to Evaluate Vutrisiran in Patients With Transthyretin Amyloidosis With Cardiomyopathy²⁶
ASO	Inotersen (IV)	...	✓	Large RCT	<ul style="list-style-type: none"> • N/A (Study in ATTR-CM currently on hold)²⁷
	AKCEA-TTR-LRx (SQ)	Ongoing RCT	<ul style="list-style-type: none"> • CARDIO-TTRransform: A Study to Evaluate the Efficacy and Safety of AKCEA-TTR-LRx in Participants With Transthyretin-Mediated Amyloid Cardiomyopathy (ATTR CM)²⁴
Transthyretin stabilizer	Tafamidis (PO)	✓	...	Large RCT	<ul style="list-style-type: none"> • Long-term Safety of Tafamidis in Subjects With Transthyretin Cardiomyopathy²⁹
	AG-10 (PO)	Ongoing RCT	<ul style="list-style-type: none"> • Open-Label Study of AG10 in Patients With Symptomatic Transthyretin Amyloid Cardiomyopathy³⁰ • ATTRIBUTE-CM: Efficacy and Safety of AG10 in Subjects With Transthyretin Amyloid Cardiomyopathy³¹
	Diflunisal (PO)	Observational	<ul style="list-style-type: none"> • N/A
Amyloid fibril disassembly	Doxycycline (PO)	Observational	<ul style="list-style-type: none"> • Tolerability and Efficacy of a Combination of Doxycycline and TUDCA in Patients With Transthyretin Amyloid Cardiomyopathy³²
	Ursodiol (TUDCA, PO)	Observational	<ul style="list-style-type: none"> • A Study of Doxycycline and Tauroursodeoxycholic Acid (Doxo/TUDCA) Plus Standard Supportive Therapy Versus Standard Supportive Therapy Alone in Cardiac Amyloidosis Caused by Transthyretin³³
	Green tea extract (PO)	Observational	<ul style="list-style-type: none"> • N/A

ASO indicates antisense oligonucleotide; ATTR, transthyretin amyloidosis; CM, cardiomyopathy; FDA, US Food and Drug Administration; hATTR, hereditary ATTR; siRNA, small interfering ribonucleic acid; N/A, Not Applicable; RCT, randomized clinical trial; and TUDCA, tauroursodeoxycholic acid.

this clinical trial, patients taking inotersen require serial monitoring of platelet counts and renal and hepatic function. In a subsequent study of patients with ATTR-CM, inotersen was shown to halt disease progression and even led to reductions in left ventricular mass on echocardiography in some patients.³ Unfortunately, a recent trial of inotersen in patients with ATTR-CM is currently on hold because of side effects of glomerulonephritis and thrombocytopenia.²⁷

Investigational Small Inhibitory RNA Molecule Therapies

In addition to intravenous small interfering RNA, several clinical trials are currently under way to study small interfering

RNA vutrisiran for treatment of ATTR. The HELIOS-A (A Study of Vutrisiran [ALN-TTRSC02] in Patients With Hereditary Transthyretin Amyloidosis [hATTR Amyloidosis]) and HELIOS-B are 2 ongoing clinical trials studying the safety and efficacy of vutrisiran in patients with hereditary transthyretin polyneuropathy and ATTR-CM (Table 2).^{25,26} A major advantage of vutrisiran is that it only requires quarterly injections in comparison to inotersen, which must be administered weekly. In addition, the phase 3 CARDIO-TTRransform (A Study to Evaluate the Efficacy and Safety of AKCEA-TTR-LRx in Participants With Transthyretin-Mediated Amyloid Cardiomyopathy [ATTR CM]) trial is currently assessing the effects of the antisense oligonucleotide molecule ION-682884 (AKCEA-TTR-LRx) on survival and major adverse cardiovascular events in patients

with ATTR-CM (Table 2).²⁸ The ION 682884 compound shares the same parent compound as inotersen but is instead conjugated to GalNAc. Unfortunately, another clinical trial of the small interfering RNA drug, revusiran, was stopped early because of increased observed mortality in the treatment arm.³⁷

Off-Label Amyloidosis Therapies

In addition to the aforementioned novel drugs, several off-label therapies have also been studied in ATTR. Expert support for these agents is mixed and their use is currently at the discretion of the treating physician.

Diflunisal is a generic nonsteroidal anti-inflammatory drug that can stabilize TTR tetramers. A randomized placebo-controlled trial demonstrated efficacy of diflunisal in reducing the rate of progression of hereditary transthyretin polyneuropathy.³⁸ A retrospective study in patients with ATTR-CM demonstrated some evidence of cardiac stabilization.³⁹

Doxycycline and ursodiol (tauroursodeoxycholic acid) were shown to disaggregate formed amyloid fibrils in *in vitro* studies.⁴⁰ In an open label study, no cardiomyopathy or neuropathy progression was observed in 7 patients after 1 year of receiving this combination.⁴¹ A retrospective study of 53 patients with ATTR-CM on this therapy showed no significant cardiac disease progression over 22 months.⁴² Larger prospective, randomized control trials are currently under way to assess the safety and efficacy of combined doxycycline and tauroursodeoxycholic acid in ATTR and ATTR-CM (Table 2).^{32,33}

Epigallocatechin 3-gallate, the most abundant catechin in green tea, demonstrated disruption of transthyretin amyloid fibrils in mouse study.⁴³ One study of 25 patients with wild-type transthyretin amyloidosis-CM treated with 600 mg epigallocatechin 3-gallate daily for 1 year showed a decrease in left ventricular mass without change in left ventricular wall thickness by CMR,⁴⁴ but ECV was not reported.

Affordability of Targeted Amyloid Therapies

With the current and expected future availability of therapies for ATTR-CM that are both efficacious and expensive, the question of whether these treatments are cost effective is highly important. Currently, the average annual costs of tafamidis, patisiran, and inotersen are a staggering \$225 000, \$350 000, and \$450 000, respectively. In cost-benefit analysis of the ATTR-ACT trial, tafamidis was not found to be cost effective at its current price with a cost per adjusted quality-of-life year of \$1 135 000.⁴⁵ Although the efficacies of patisiran and inotersen for ATTR-CM are not yet known, similar concerns regarding cost effectiveness are likely to occur at their current prices. This creates an ethical dilemma wherein the

equitable delivery of potentially life-prolonging therapies may be limited by drug costs. This has profound implications when one accounts for the increasing recognition of the high prevalence of ATTR-CM.⁴⁻⁶ It is therefore likely that either reductions in drug prices or the development of less expensive novel agents will be necessary before targeted ATTR-CM therapies can become widely available.

EMERGING CHALLENGES AND DILEMMAS

CA is diagnosed and staged using a multimodality approach combining cardiac imaging, biomarkers, and occasionally invasive tissue biopsy. Echocardiography and CMR have an important role in screening patients suspected to have CA based on clinical features. ECV by CMR is highly reproducible and can track amyloidosis disease burden serially to assess therapeutic response.^{10,46,47} Tc-PyP/DPD and light chain analysis are important in screening for CA and in helping to determine amyloid subtype. Invasive tissue biopsy is required in immunoglobulin light chain; however, in ATTR, invasive biopsy is generally reserved when the initial testing is inconclusive. While this approach successfully guides therapy for most patients, there remain key gaps in our current understanding. These include growing awareness that indeterminate or discrepant findings can be seen with either cardiac imaging studies or blood biomarkers, and can complicate the diagnosis of CA. Additionally, the optimal method of screening asymptomatic ATTRv carriers and monitoring response to amyloid treatment is currently poorly understood.

Interpreting Indeterminate Cardiac Imaging

While echocardiography and CMR are highly valuable modalities in the evaluation of suspected CA, each has its limitations. Many of the studies that established the high diagnostic accuracy of CMR and echocardiography were performed at referral centers or in patients with classic CA, introducing the possibility of referral bias and questions on sensitivity among more heterogeneous populations and community-based healthcare systems. It is unclear whether these tests would be as accurate in routine clinical practice where the prevalence of CA is much lower. Echocardiography in particular can be negative in early-stage CA. In a small study of 12 asymptomatic ATTRv carriers without evidence of CA on echocardiogram, Tc-PyP uptake was still seen in 84% of cases.⁴⁸ Additionally, echocardiography cannot reliably differentiate CA from other causes of

pathologic left ventricular hypertrophy such as hypertensive heart disease, hypertrophic cardiomyopathy, and other infiltrative disorder that must be carefully excluded.

While CMR is more sensitive than echocardiography given its tissue characterization capabilities, there are currently a lack of definitive data comparing these 2 modalities in ATTR. Although elevated myocardial ECV and diffuse LGE on CMR are classical features of CA, these may not be present in early-stage disease. In early CA, only faint LGE and mild elevations in ECV may be present, which may sometimes make it difficult to differentiate from myocardial fibrosis (eg, from hypertensive heart disease). Mild elevations in ECV and LGE may also be seen in hypertensive heart disease because of the presence of diffuse and focal fibrosis.⁴⁹

In our clinical practice, we find echocardiography and CMR to be highly useful but not infallible screening tools in patients suspected to have CA. When positive, these tests can reliably guide further testing. When pre-test probability is low, an unremarkable echocardiogram and/or CMR is often sufficient to exclude CA. However, because of the potential for lack of sensitivity, when pre-test probability is high, we frequently pursue Tc-PyP/DPD and light chain analysis, even when CMR or echocardiography appear normal to detect the earliest stages of disease where early treatment may matter most.

Interpreting Indeterminate Findings in Amyloid Subtyping

In some cases, it may be difficult to determine amyloid subtype based on the results of the initial testing. For example, ATTR-CM cannot be diagnosed noninvasively with Tc-PyP/DPD when a monoclonal serum or urine light chain is present. Up to 20% of patients with AL-CM may have false-positive uptake on Tc-PyP/DPD.¹⁴ In fact, the most common error in diagnosing CA is failure to exclude AL-CM when Tc-PyP/DPD uptake is present.⁵⁰ Serum protein electrophoresis alone is insufficient to rule out AL-CM and may be negative in more than half of cases.⁵¹ Light chain analysis should include serum free light chains and both serum and urine protein electrophoresis with immunofixation. This combination can reliably exclude AL-CM with 99.8% certainty.⁵¹ Additionally, 10% of patients with ATTR-CM also have an incidental monoclonal gammopathy of undetermined significance unrelated to their CA diagnosis.⁵² Therefore, when both grade ≥ 2 uptake on Tc-PyP/DPD and monoclonal light chain are both present, invasive biopsy is required to establish subtype.

One must also be careful to avoid pitfalls in interpreting Tc-PyP/DPD scans. A common cause of false-positive Tc-PyP/DPD and referrals to amyloid centers

is blood pool uptake. High concentrations of Tc-PyP/DPD in the blood pool can mimic myocardial uptake on planar imaging and may occur particularly in low cardiac output states.⁵⁰ This can be excluded with the use of single photon emission computed tomography imaging to confirm that uptake is of myocardial and not blood pool origin. False-positive Tc-PyP scans have also been reported to occur with cardiotoxicity from hydroxychloroquine.⁵³ While the sensitivity for Tc-PyP appears to be superior to echocardiography for detecting ATTR-CM, false negatives can also occur.⁴⁸ For example, of the 111 patients with grade 0/1 uptake on Tc-PyP/DPD in the Gilmore et al study, 18% were ultimately found to have ATTR-CM on biopsy.¹⁴ Among patients with just grade 1 uptake on Tc-PyP/DPD, the prevalence of ATTR-CM was even higher at 46%. False-negative scans can also occur, particularly in patients with earlier cardiac involvement or in those with specific mutations such as Phe64Leu and Val30Met.^{50,54} Therefore, one should be somewhat careful in excluding ATTR-CM solely on the basis of Tc-PyP/DPD, particularly when grade 1 uptake is present and a high clinical suspicion remains.

At our institution, when findings on Tc-PyP and light chain analysis are indeterminate, we generally proceed with endomyocardial biopsy. When pre-test probability is high, we may sometimes proceed with endomyocardial biopsy even despite the absence of Tc-PyP uptake, given the possibility of false-negative results, particularly with grade 1 uptake. In some instances, we may also opt to follow patients with a repeat CMR or Tc-PyP scan 6 to 12 months later to evaluate for the subsequent development of cardiac involvement.

Surveillance of the Asymptomatic ATTRv Carrier

Genetic testing is indicated in all patients diagnosed with ATTR-CM to screen for a pathologic gene mutation (ATTRv). The most commonly identified pathologic ATTR gene mutations worldwide are Val30Met, Val122Ile, and Thr60Ala.⁵⁵ Genetic testing has implications not just for the patient but also for family members in whom identification of a pathologic ATTRv mutation can help facilitate early diagnosis and treatment. In patients with ATTR-CM, initiation of therapy at an earlier stage of illness is associated with better outcomes. In subgroup analysis of the ATTR-ACT study, tafamidis demonstrated net benefit in both survival and cardiac hospitalizations among patients with baseline New York Heart Association class I–II heart failure symptoms.¹ Conversely, in those with New York Heart Association Class III symptoms, increased rates of hospitalizations were observed in the tafamidis-treated group. Based on these data, the US Food and Drug Administration has approved the use of tafamidis

for all patients with ATTR-CM regardless of symptoms. Based on this evidence, a large multimodality imaging consensus statement considered it reasonable to screen asymptomatic carriers of pathologic ATTRV gene mutations for cardiac involvement.¹⁵

Currently, the ideal method and intervals for screening ATTRV carriers remain unknown. Yet, it may be reasonable to screen ATTRV carriers before the expected age of onset of cardiomyopathy for their particular mutation. For patients with the Val122Ile, Val30Met, and Th60Ala mutations, onset of cardiomyopathy is typically beyond 65, 50, and 45 years, respectively.⁵⁶ In comparison, cardiomyopathy usually occurs later in wild-type transthyretin amyloidosis beyond the sixth decade of life.

ATTRV carriers may be screened for cardiac involvement with serum biomarkers, electrocardiography, echocardiography, CMR, and/or Tc-PyP/DPD. Biomarkers such as NT-proBNP and troponin are an attractive choice because they are inexpensive and widely available. NT-proBNP levels appear to be higher in ATTRV carriers with cardiac involvement as compared with asymptomatic ATTRV carriers.^{48,57} An ECG may be obtained to screen for low-voltage pattern. However, both biomarkers and ECG lack sensitivity and may be negative, particularly in early-stage disease.^{48,58}

Echocardiography and CMR have been demonstrated to have excellent sensitivity and specificity in symptomatic patients; however, there is a paucity of data assessing their use in screening ATTRV carriers. The modality with the most robust evidence in support of screening in ATTRV carriers appears to be Tc-PyP/DPD. Among ATTRV carriers, cardiac involvement is frequently observed using Tc-PyP and may precede echocardiographic evidence of disease by 1 to 2 years.^{48,59} One concern with repeated Tc-PyP scans is the theoretical risk of cancer related to repeated exposure to ionizing radiation. While the risk associated with a single Tc-PyP scan appears to be low, the effects of serial scans—particularly in younger patients—is currently unknown.⁶⁰

Despite this, based on the available evidence, we generally begin screening ATTRV carriers 5 to 10 years before the expected age of onset of cardiomyopathy based on their gene mutation and family history. We typically screen all ATTRV carriers initially referred to our clinic with troponin, NT-proBNP, ECG, a Tc-PyP scan, an echocardiogram, and frequently a CMR. When cardiac involvement is not present, we often repeat biomarkers, an ECG, and either an echocardiogram or a Tc-PyP scan annually. When a strong family history of cardiomyopathy or indeterminate imaging findings such as grade I uptake on Tc-PyP are present, we preferentially perform surveillance with a Tc-PyP/DPD scan given its superior sensitivity when

compared with echocardiography. In our comprehensive amyloid clinic, we also work collaboratively with hematology, nephrology, and neurology departments to monitor for signs of other end-organ manifestations such as neuropathy and nephropathy.

Monitoring Response to Therapy

Another current gap in our understanding is how best to monitor response to therapy in patients receiving treatment for CA. Multiple markers may be used for this purpose including clinical parameters, cardiac biomarkers, and cardiac imaging. There is evidence from recent clinical trials to support all of these methods.

Functional capacity and patient quality of life appear to be impacted by treatment of ATTR-CM.¹ In the ATTR-ACT study, tafamidis was found to attenuate declines in both 6-minute walk test and Kansas City Cardiomyopathy Questionnaire as compared with placebo.¹ One criticism of patient-centered outcomes such as the Kansas City Cardiomyopathy Questionnaire is that they are nonspecific. It may therefore be difficult to determine whether improvements in these markers are because of changes in cardiac, nerve, or other end-organ functioning. As a result, their role in clinical practice is currently uncertain. Improvements or stabilization in cardiac biomarkers such as NT-proBNP have also been observed in patients with ATTR-CM treated with patisiran and tafamidis.^{1,36}

With respect to cardiac imaging, there is evidence that echocardiography, CMR, and positron emission tomography (PET) imaging may all potentially be useful. In large randomized studies, echocardiographic parameters including left ventricular mass and wall thickness have been shown to differ between patients with ATTR-CM treated with patisiran and inotersen as compared with placebo.^{36,61} However, these measurements may be less reproducible because of the lower spatial resolution of echocardiography and the reliance on geometric assumptions to calculate left ventricular mass. CMR-derived ECV is a useful marker for trending treatment response in CA since ECV quantifies the degree of expansion of the interstitium where amyloid protein resides. In patients with ATTR-CM, ECV has been shown to decrease in response to patisiran treatment.⁴⁷ Similarly, in AL-CM, reductions in ECV have been demonstrated in response to chemotherapy and are associated with favorable treatment response.^{10,46} For these reasons, CMR tissue characterization parameters (eg ECV) are increasingly being incorporated in therapeutic clinical trial outcomes. Because it is the most quantitative, highly reproducible marker available, the use of ECV should be strongly considered to monitor responses to therapies, particularly in the context of research studies.

Although there is a growing body of literature regarding the quantification of bone-avid radiotracers using calcium zinc telluride–single photon emission computed tomography, serial Tc-PyP/DPD are not appropriate for monitoring response to therapy.^{19,20} In a study of patients with ATTR-CM, neither heart-to-contralateral lung ratio nor visual uptake changed significantly over time despite obvious clinical deterioration.¹⁸ More recently, novel PET tracers have been developed, which have been shown to bind with high avidity to both ATTR and immunoglobulin light chain deposits including the ¹¹C-Pittsburgh B (¹¹C-PiB) and ¹⁸F-Florbetapir compounds.^{62,63} Unlike Tc-PyP/DPD, PET tracer uptake can be quantified and correlates closely with amyloid burden.⁶² Because of this, PET uptake with ¹¹C-Pittsburgh B (¹¹C-PiB) and ¹⁸F-Florbetapir may be a promising way to quantify response to therapy in CA. The sensitivity of ¹⁸F-Florbetapir for detecting early cardiac involvement in immunoglobulin light chain amyloidosis may even exceed that of CMR.⁶⁴ However, the higher cost and radiation exposure with cardiac PET as compared with CMR are potential limitations to its widespread use. A prospective clinical trial is already under way to track changes in ¹⁸F-Florbetapir uptake in patients receiving chemotherapy for AL-CM.⁶⁵

To date, the optimal marker for assessing response to treatment in CA remains unclear. Aside from the use of ECV in AL-CM treatment, it is also currently unknown whether changes in serial markers can predict treatment response. Future studies are needed to identify noninvasive markers, which can predict response to treatment in order to best tailor pharmacotherapy to each individual patient with CA. In the clinical setting, we generally follow patients with annual troponin, NT-proBNP, ECG, and an echocardiogram while being treated for ATTR-CM because they are affordable and pose no risk to the patient.

METHODS TO ADDRESS DIAGNOSTIC CHALLENGES IN THE FIELD

While echocardiography, CMR, and Tc-PyP/DPD have an established role in imaging CA, there remain gaps in our understanding of how best to apply these modalities. For example,

while the diagnostic efficacies of echocardiography, CMR, and Tc-PyP/DPD have been well studied in patients with suspected CA, few studies have directly compared them. Further multimodality studies are needed to determine the cardiac imaging modality of choice for screening patients with suspected CA and asymptomatic ATTRv carriers. Additionally, further studies are needed to better understand the role of novel cardiac imaging techniques such as

¹⁸F-Florbetapir PET, and hybrid PET/CMR.^{65–69} Studies are also currently under way to assess whether serum misfolded ATTR oligomer levels can be used to non-invasively screen for cardiac involvement in ATTR.⁷⁰ Whether these contemporary techniques have a role in clinical practice or are better suited to the research setting is currently unknown.

Moreover, further research is needed to determine the ideal cardiac imaging and blood-based biomarkers for assessing response to therapy in CA both in clinical practice as well as clinical trials. While ongoing clinical trials dedicated to this question are limited, cardiac imaging markers are now increasingly being utilized as end points in large pharmaceutical trials.²⁶ The increased inclusion of echocardiography, CMR, and PET parameters in amyloid clinical trials will help us to better understand the additive role of cardiac imaging in monitoring treatment response in CA.

OTHER ONGOING PHARMACEUTICAL TRIALS

In addition to tafamidis, there are now a number of active clinical trials testing both new drugs and novel applications of existing treatments for ATTR-CM. As discussed above, multiple studies—including the APOLLO-B, HELIOS-B, and CARDIO-TTRransform trials—are currently under way to assess the efficacy of inhibitory oligonucleotide therapies including patisiran, vutrisiran, and ION-682884 (AKCEA-TTR-LRx) in patients with ATTR-CM.^{23,26,28} Additionally, Phase III clinical trials are currently under way to test the novel stabilizing molecule acoramidis (AG-10) in patients with ATTR-CM.³¹ A comprehensive list of active drug trials is summarized in Table 2. Hopefully, trials such as these will result in the availability of additional, effective therapies to treat patients with ATTR-CM. These trials, combined with advancements in cardiac imaging and clinical experience, will enhance our ability to effectively identify and treat even less advanced forms of CA.

CONCLUSIONS

The development and approval of multiple agents that stabilize or prevent production of transthyretin protein has greatly improved outcomes in both hereditary transthyretin polyneuropathy and cardiomyopathy. Over the same period, diagnostics in the field of CA have also substantially improved. Echocardiography, CMR, and Tc-PyP have become established tools in the diagnosis and management of CA. Novel cardiac imaging techniques including myocardial PET may also have an evolving role in CA. Hopefully in the

future, we will have a better understanding of how each of the biomarkers and cardiac imaging modalities can best complement each other in the diagnosis, risk stratification, and monitoring of treatment in ATTR-CM. While it is difficult to imagine how the speed of recent monumental gains made in the field of ATTR-CM could be matched in the future, with an increasing number of new drugs in development as well as novel applications of cardiac imaging and biomarkers, the future appears similarly promising. With the inclusion of more precise strategies for the diagnosis, prognostication, and management of ATTR-CM, we are poised for dramatic alterations to the care of these patients.

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