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Pathophysiology and Therapeutic Approaches to Cardiac Amyloidosis

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Abstract

Often considered a rare disease, cardiac amyloidosis is increasingly recognized by practicing clinicians. The increased rate of diagnosis is in part due the aging of the population and increasing incidence and prevalence of cardiac amyloidosis with advancing age, as well as the advent of non-invasive methods using nuclear scintigraphy to diagnose transthyretin cardiac amyloidosis due to either variant or wild type transthyretin without a biopsy. Perhaps the most important driver of the increased awareness is the elucidation of the biologic mechanisms underlying the pathogenesis of cardiac amyloidosis which have led to the development of several effective therapies with differing mechanisms of actions. In this review, the mechanisms underlying the pathogenesis of cardiac amyloidosis due to light chain (AL) or transthyretin (ATTR) amyloidosis are delineated as well as the rapidly evolving therapeutic landscape that has emerged from a better pathophysiologic understanding of disease development.

Keywords

Amyloidosis; Cardiac; Pathogenesis; Therapy; cardiomyopathy; treatment; pathophysiology; Transthyretin; immunoglobulin light chains amyloid

Subject Terms:

Cardiomyopathy; Heart Failure; Pathophysiology; Treatment

The elucidation of physiologic mechanisms underlying the genesis of misfolded proteins which form amyloid fibrils that deposit in the myocardium and can cause cardiac amyloidosis has led to development of several effective therapeutic approaches¹. These efforts have led to therapies that have been described as a “translational triumph”². Among the causes of cardiac amyloidosis, the two that account for >95% of cases encountered clinically (Table 1) include: (1) immunoglobulin light chain (AL) cardiac amyloidosis, which is due to a plasma cell dyscrasia with over-production of either kappa or lambda light chains, and (2) transthyretin (TTR) cardiac amyloidosis, which results from misfolded monomers or oligomers of either wild type (ATTRwt) or variant transthyretin (ATTRv)

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cardiac amyloidosis³. ATTRv is inherited in an autosomal dominant fashion and is due to one of the more than 130 mutations in the transthyretin gene on chromosome #18. With the aging of the population, ATTRwt cardiac amyloidosis (CA) is anticipated to become the most common form of systemic amyloidosis. In this review, we will delineate the mechanisms underlying the pathogenesis of cardiac amyloidosis and highlight the rapidly evolving therapeutic landscape that has emerged from a better understanding of disease development.

Pathophysiology

Despite originating from different precursor proteins, the basic mechanisms underlying amyloid pathogenesis is similar in that the capability of a protein to become amyloidogenic lies in its ability to acquire more than one conformation. Amyloid formation occurs when a protein loses (or fails to acquire) its physiologic, functional fold. A number of factors may trigger protein misfolding and aggregation, such as abnormal proteolysis, point mutations and post-translational modifications such as phosphorylation, oxidation and glycation. The misfolded protein or peptide then assembles with similar proteins or peptides to form oligomers, which circulate in the blood and deposit as highly ordered fibrils in the interstitial space of target organs. In cardiac amyloidosis, the mechanisms of organ dysfunction are likely multifactorial, resulting from a combination of factors including extracellular deposition of amyloid in the parenchymal tissue leading to mechanical disruption of tissue structure, as well as proteotoxicity of the fibrils or pre-fibrillar proteins leading to inflammation, reactive oxygen species generation, apoptosis and autophagy, which can be observed even prior to fibril deposition⁴⁻⁷. This leads to a restrictive physiology, diastolic dysfunction and eventually manifests clinically as heart failure.

Overview of protein folding

Protein or peptide folding is a tightly regulated process. In general, proteins require specific three-dimensional conformations in order to be soluble and function correctly in the body. The process of protein folding begins after polypeptide chains are synthesized in the endoplasmic reticulum of the cell and a rapid sequence of intracellular folding consisting of conformational modifications is initiated, requiring the use of chaperones and catalysts of folding, to achieve its native structure. In this pathway, conformational intermediates become progressively more organized as they merge, resulting in the most stable native state. In this native structure, there is a minimum of free energy which results from the balance between the internal energy of the protein determined by intramolecular bonds and the level of conformational entropy, determined by the level of randomness of the polypeptide in solution⁸.

Proteins must remain folded throughout their lifetimes to continue to perform their biological functions and the abundance of each of the thousands of different proteins in a cell must be tightly regulated. This state of a balanced proteome, termed protein homeostasis or proteostasis, requires an extensive network of competing pathways within cells that control the protein synthesis and folding, conformational maintenance and degradation of proteins present within and outside the cell⁹. The proteostasis network

serves to ensure that correctly folded proteins are generated at the proper time and cellular location and in amounts allowing stoichiometric assembly in the case of oligomeric protein complexes. Additionally, it prevents proteins from misfolding and aggregating, and removes proteins that are misassembled. Normally, misfolded proteins are retained by the endoplasmic reticulum, dislocated to the cytoplasm and degraded by the proteasome¹⁰. Loss of proteostasis is the underlying cause of disease associated with protein misfolding, such as amyloidosis.

Mechanisms of amyloidogenesis.

Folded protein structures, in most cases, are only marginally stable, meaning that a substantial proportion of protein species exist in unfolded states. The exposure to various extra-cellular denaturing stimuli causes unfolding of the polypeptide chain, an event which is normally followed by the rapid restoration of the native structure. This extra-cellular unfolding and refolding process causes the exposure of normally hidden hydrophobic residues and the protein may become the target of ubiquitous endopeptidases. Even minor proteolytic cleavage can destabilize the protein, promote its denaturation and prevent the restoration of the native structure.

Partial unfolding of the native state of the protein to less thermodynamically stable states is a required step in amyloidogenesis¹¹. Amyloidogenic and normal protein counterparts are synthesized, but cellular quality control appears to be unable to remove misfolded proteins and they are secreted from the cell¹¹. Outside the cell, amyloidogenic variants reach a state of equilibrium between fully folded and partially folded forms. Any factor that disrupts the normal three-dimensional protein structure, such as low pH, oxidation, increased temperature, can shift this equilibrium towards the partially folded state. A misfolded protein must then reach critical local concentration to trigger fibril formation, in conjunction with local factors including glycosaminoglycans and collagen, shear forces, endoproteases and metals that modulate aggregation and oligomer formation^{9, 12, 13}.

In both ATTRv and ATTRwt, amyloid aggregation of TTR is preceded by destabilization of the native homotetrameric structure into its constituent monomers and dimers with an exposed hydrophobic surface, followed by misfolding and structural reorganization into amyloid aggregates (Figure 1). Physiologic TTR is a homotetramer, mainly synthesized in the liver and the choroid plexus of the brain, circulates in plasma and CSF and serves as a carrier of thyroxine and retinol bound to retinol-binding protein¹⁴. TTR consists of 2 weakly bound dimers, between which lie 2 thyroxine (T₄) binding sites. It is at these sites that the dimers of TTR dissociate and the process of destabilization and unfolding occur. In ATTRv, different mutations lead to a kinetically unstable tetrameric protein with an increased propensity to dissociate into monomers leading to misfolding¹⁵. For example, the Val122Ile (p.Val142Ile), variant destabilizes the TTR tetramer by lowering the kinetic barrier for tetramer dissociation, resulting in a greater extent and faster rate of folded monomer formation that then self assembles into amyloid fibrils *in vitro*. Despite structural instability, mutant TTR tetramers are secreted with the same efficacy as wild type if they possess a thermodynamic and kinetic profile to escape the endoplasmic reticulum degradation pathway. In ATTRwt, which is associated with increased age, protein oxidative

modifications and failures in the proteostatic machinery and repair mechanisms associated with aging, contribute to native TTR dissociation and aggregation into fibrils⁵.

AL amyloidosis is characterized by a clonal expansion of differentiated plasma cells, that produce misfolding-prone immunoglobulin free light chains, or a fragment thereof, secreted in excess compared to heavy chains^{14, 16}. The two classes of light chains, kappa and lambda, each consist of an N-terminal variable Ig domain attached to a C-terminal constant Ig domain. While excess free light chain production is observed in plasma cell disorders including monoclonal gammopathy of undetermined significance, multiple myeloma and Waldenstrom macroglobulinemia, only a fraction of free light chains can form amyloid deposits in vivo. Lambda light chains are observed almost twice as commonly as kappa in systemic AL amyloidosis¹⁷. DNA sequencing studies have shown presence of germline gene mutations on the variable lambda region, V λ 6a and V λ 3r, that reduce the thermodynamic stability of the protein, have a strong association with the development of amyloidosis and account for the propensity of lambda light chains to form amyloid deposits¹⁸. Amyloidogenic light chains are kinetically unstable and susceptible to endoproteolysis, which results in the release of amyloidogenic light chain fragments prone to improper aggregation¹⁹.

Other less commonly forms of cardiac amyloidosis encountered clinically include AA amyloidosis, dialyses associated amyloidosis and isolated atrial amyloidosis. AA amyloidosis is associated with auto inflammatory condition such as rheumatoid arthritis, inflammatory bowel disease and hidradenitis suppurativa, particularly when the diagnosis is delayed. Serum amyloid associated protein (SAA) is elevated and cardiac involvement in AA amyloidosis is always preceded by renal involvement. Effective control of the underlying inflammatory process can halt disease progression and even reverse organ damage.²⁰ β 2-microglobulin is the precursor protein for cardiac amyloidosis associated with long term dialysis, Cardiac amyloidosis related to β 2-microglobulin occurred with low-flow dialysis membranes during dialysis of > 9 years in duration. However, newer dialysis technologies reduce serum β 2-microglobulin levels in chronic dialysis patients and appear to reduce the risk of developing this form of systemic amyloidosis.²¹ Isolated atrial amyloidosis (IAA), due to deposits of amyloidosis from atrial natriuretic factor, is extremely common with advancing age and often seen on biopsies of the atrium obtained at the time of cardiac surgery.²² Distinguishing these forms of amyloidosis from those more commonly encountered clinically is essential.

Amyloid fibril structure

Despite originating from different precursor proteins, amyloid deposits share several structural properties as observed by electron microscopy. They are composed of rigid, non-branching fibrils with an average diameter of 7.5–10nm and a cross- β -sheet secondary structure. Intermolecular main-chain hydrogen bonding between the amide and carbonyl groups of the main chain acts as a major stabilizing interaction between protein monomers^{8, 23} that allow formation of the β -sheet. Both immunoglobulin light chains and TTR protein have extensive β -structure in the normal folded state, but this region has to be exposed for intermolecular hydrogen bonding between monomers. Contiguous β -sheet

polypeptide chains constitute a protofilament, which are wound around one another to form an amyloid fibril, which frequently have repetitive hydrophobic or polar interactions along the fibril axis²³. This ultrastructure of the fibril allows the intercalation of Congo red dye, conferring the diagnostic property to amyloid of apple-green birefringence under polarized light microscopy.

Beside the fibril core proteins, additional components are known to be part of all amyloid deposits including components of the extracellular matrix, such as lamin, entactin and collagen, and additional proteins. Serum amyloid P-component (SAP), a glycoprotein that belongs to the pentraxin family, is calcium dependently bound to amyloid fibril independently of the protein of origin²⁴. SAP is highly protected against proteolysis²⁵, making amyloid fibrils highly rigid, resistant to thermal and chemical denaturation and degradation. Proteoglycans or glycosaminoglycans are also common in amyloid deposits and contribute to the carbohydrate composition of amyloid, influencing the conformation of the fibril. Their role is quite complex and seem to contribute to both the genesis and the structural stabilization of amyloid fibrils. Proteoglycans are proposed to represent the initial structural scaffold that facilitates adhesion and orientation of the first nuclei of aggregated amyloid. Despite being common component of all amyloid deposits, proteoglycans show a degree of chemical and structural heterogeneity, depending on the tissue and may play a role in the localization of amyloid deposits in tissues.

Amyloid deposition

Amyloid deposition in target organs occurs by an extremely complicated aggregation process. The kinetics of fibril formation has an S-shaped growth curve and a discernable lag phase. The duration of each phase of fibril formation is protein specific and defines the rate at which amyloid deposition occurs²⁶. First, primary nucleation occurs when soluble oligomers form from monomers. The initial nucleation process is driven by specific adhesive parts of the amyloid proteins and target organ cells may be transiently involved¹¹. In ATTR, two adhesive segments that form the F and H β -strands in the native TTR structure are the principal drivers of protein aggregation. Upon dissociation into monomers, these strands become exposed and enable stacking into the steric zipper spines of amyloid fibrils²⁷. Circulating monomers can then add to this existing fibril. Secondary nucleation occurs when the surface of this existing amyloid aggregate catalyzes the formation of new small soluble aggregate and fragmentation occurs when the existing fibrils break apart, increasing the total number of fibrils. The process of amyloid deposition can be accelerated by the presence of preformed fibrils, or seeds, which can capture and catalyze the conversion of precursors, even at low concentrations, into misfolded, toxic and aggregation-prone structures²⁸.

Deposition of amyloid in specific organ tissues likely depends on the concurrence of several factors including high local protein concentrations, low pH and the presence of fibril seeds. Specific interactions with tissue glycosaminoglycans or cell surface receptors may be important²⁹. In AL amyloidosis, it has been hypothesized that organ tropism may be a function of the variable region gene polymorphisms, leading to interactions between the light chain (or fragment thereof) and tissue constituents such as collagen, lipids and glycosaminoglycans¹⁹. For example, LV1-44 germ line cells favor deposition in cardiac

tissue¹⁸. In ATTRv, the specific site of amino acid substitution determines the propensity for depositing primarily in the peripheral nervous system or cardiac tissue, leading to markedly different phenotypes of disease. Patients with the Val122Ile (p.Val142Ile) TTR variant present predominantly with a cardiomyopathy⁴ whereas other variants such as Val30Met (p.Val50Met) are associated with neuropathy. While for certain mutations the correlation between the genotype and phenotype is strong, for others clinical features can vary significantly. Furthermore, significant variability in clinical presentation is seen between patients with the same mutation. Cleavage of the TTR monomer into fragmented fibrils may play a role as well in determining the site of deposition as well as the disease penetrance³⁰. Full length TTR fibrils are commonly seen in ATTRv caused by the Val30Met (p.Val50Met) variant with early disease and a predominant axonal polyneuropathy with rare cardiac involvement, whereas those with later onset disease often have fibrils composed of a mixture of full-length and truncated TTR. These patients also often have marked cardiac involvement at presentation with concomitant peripheral neuropathy. Similarly, in patients with ATTRwt, amyloid deposits always include both fragmented and full length TTR fibrils³¹. Indeed, environmental and genetic factors that have not been identified must play a role in the pathobiology of TTR amyloidosis.

Mechanisms of cardiac dysfunction (Figure 2)

The deposition of amyloid fibrils results in cellular injury, tissue damage and finally organ dysfunction. Although the type of cardiac amyloidosis cannot be distinguished based on patterns of deposition, it appears there is a predominance of diffuse, peri-cellular, endocardial and arterial or arteriolar deposits in AL amyloidosis and nodular deposits in TTR amyloidosis³². In both AL and TTR cardiac amyloidosis, large deposits of amyloid in the extracellular space of the myocardium leads to loss of normal tissue architecture and function, progressive biventricular wall thickening and stiffness without compensatory ventricular dilation, leading to a restrictive myopathy and low cardiac output¹¹. Early disease is marked by isolated diastolic dysfunction with normal systolic function but as the disease progressive restrictive physiology becomes apparent, atrial infiltration is present and frequently causes contractile dysfunction. Insights from non-invasive pressure-volume analysis in patients with both wild-type and Val122Ile associated TTR-CA demonstrate a complex cascade of events occurring overtime marked by decreasing ventricular capacitance and chamber contractility leading to reduced stroke volume, alterations in ventricular-vascular coupling and progressive pump dysfunction not simply due to impairment in diastolic dysfunction but systolic derangements as well³³ (Figure 3).

Mechanical displacement of normal parenchymal tissue by amyloid deposits is insufficient to fully explain organ dysfunction associated with both AL and TTR amyloidosis (Figure 2). In addition to the mechanical problems imposed by amyloid fibril deposition, small soluble monomers and oligomers are extremely toxic and believed to play a major role in cell and tissue toxicity. The direct toxic effect of circulating light chains in AL amyloidosis has been postulated to explain discrepancies between myocardial amyloid fibril burden, cardiac dysfunction and the more aggressive disease trajectory in those with AL compared with TTR cardiac amyloidoses³⁴. Notably, not only amyloid deposition, but light chain proteotoxicity exhibits specific organ tropism⁷. Pre-fibrillar cardiotoxic

light chains alter cellular redox state in cardiomyocytes, marked by an increase in intracellular reactive oxygen species (ROS), increased apoptosis and cause oxidative stress⁷. Oxidant stress imposed by the light chains result in direct impairment of cardiomyocyte contractility and relaxation with associated alterations in intracellular calcium handling^{4, 35}. The activation of p38 mitogen-activated protein kinase (MAPK) is one of the molecular mechanisms responsible for cardiotoxicity by increasing oxidative stress and apoptosis. This pathway also mediates type B natriuretic peptide transcription, supporting the association between cardiotoxic light chain effects with induced MAPK signaling and elevated brain natriuretic peptide (BNP) levels³⁶. Indeed, the degree of circulating light chain abnormality is clinically prognostic in patients with AL amyloidosis³⁷, and correlates with cardiac biomarker elevations. Furthermore, reduction in circulating amyloidogenic free light chain concentrations by chemotherapy translates into reductions in BNP, despite unaltered amyloid deposition in the myocardium³⁸.

In TTR amyloidosis, an accumulating body of evidence suggests that tissue dysfunction precedes TTR fibril deposition, suggesting as well that circulating pre-fibrillar proteins cause toxicity. In vitro, TTR monomers and oligomeric intermediates smaller than 100kDa, but not large aggregates or amyloid fibrils, induce cytotoxicity through interactions with membrane proteins and cholesterol⁷. Apoptotic mechanisms are activated through cleavage of caspase 3/7 and superoxide formation^{4, 6}. However, the relevance of these short-term in vitro findings to disease that manifest clinically over months to years remains unclear⁶.

Presentation and diagnostic evaluation

Systemic manifestations of TTR deposition, such as carpal tunnel syndrome, lumbar spine stenosis or biceps tendon rupture, may precede cardiac diagnosis of ATTR-CA by several years, survival after which is approximately 2–5 years without treatment. In contrast, AL-CA is a more rapidly progressing disease, with a median survival of 6 months from the onset of heart failure if untreated. Although a detailed description of the presentation and diagnostic evaluation of cardiac amyloidosis is outside of the scope of this review, table 2 outlines the cardinal manifestations, approach to diagnostic testing and characteristic findings for both AL- and ATTR-CA.

Therapeutic targets

Emerging from the basic molecular mechanisms of the genesis of amyloid fibrils in the myocardium that underlie the development of cardiac amyloidosis, there are several therapeutic strategies that have either been shown to be effective or are actively being explored in human clinical trials. These approaches broadly include one of four strategies including:

1. knocking down production of the precursor protein with either gene silencing techniques for TTR that leverage small interfering RNA or anti-sense oligonucleotides (both of which include approved compounds for ATTRv disease with or without a concomitant cardiomyopathy) and CRISPR based approaches; for AL amyloidosis there is a large and growing armamentarium of anti-plasma cell therapies.

2. Stabilization of the precursor protein in order to maintain its normal conformational structure which has led to the development of tafamidis for TTR cardiac amyloidosis³⁹ and the investigation of other TTR stabilizers (e.g. AG10), as well the identification of small molecules that kinetically stabilize light chains.⁴⁰
3. Degradation/Disruption of amyloid fibrils with use of monoclonal antibodies targeted at particular epitopes on misfolded and/or aggregated proteins that either induce macrophage mediated dissolution or disruption of amyloid formation^{41–43}
4. Anti-seeding therapies that involve peptides designed as inhibitors to cap fibril growth.^{44, 45}

Effective and Emerging Therapies Based on Biologic Mechanisms

Although ATTR and AL amyloidosis both result in the deposition of amyloid fibrils and damage to the involved organs, treatment regimens are distinct. Therapies are generally more effective if administered before significant cardiac dysfunction has ensued (Figure 4). The following sections will describe:

1. Reducing the precursor protein or stabilization of TTR amyloid in cardiac amyloidosis.
2. Anti-plasma cell therapies for the treatment of AL amyloidosis.
3. Agents targeting the degradation and/or extraction of TTR or AL amyloid fibrils.
4. Emerging therapies for ATTR and AL amyloidosis.

Knocking Down or Reducing Precursor Production (Table 3).

There are 2 classes of gene silencer therapy for TTR amyloidosis currently commercially available or in late phase clinical trials. The first, small interfering RNA (siRNA) molecules and the second, antisense oligonucleotides (ASOs). These molecules knockdown or reduce TTR production, but with slightly different mechanisms. Vitamin A supplementation must be provided in those receiving silencer therapy given that a major function of TTR is to transport retinol, the major circulating form of vitamin A, via retinol binding protein 4. Thyroxine supplementation is not necessary given the fact that the majority (~85%) of thyroxine is bound to thyroxine binding globulin or albumin.

First Generation Therapies: Inotersen (*Tegsedi*, Akcea Therapeutics, Inc), an ASO, is a 2'-O-methoxyethyl-modified antisense oligonucleotide inhibitor of TTR production in the liver. It is currently approved in the US for treatment of stage 1 or 2 polyneuropathy due to ATTRv. ASOs⁴⁶ are single stranded, amphipathic DNA sequences which have a high binding affinity for proteins, thus enhancing distribution in the body. ASOs are taken up into the liver by binding with hepatocyte surface proteins, mainly clathrin- or caveolin-mediated uptake⁴⁶, and subsequently transported to the nucleus by chaperone proteins. It is here in the nucleus that the ASO binds to the target mRNA protein and via the endonuclease, RNase H2, initiates mRNA degradation. The 2'-O-methoxyethyl modification provides

resistance against endogenous degradation of the ASO. The NEURO-TTR trial randomized 172 patients with familial amyloid polyneuropathy with or without ATTR-CA to inotersen 300mg delivered subcutaneously weekly or placebo for 64 weeks. Subjects with New York Heart Association (NYHA) class III or greater were excluded from participation. During the NEURO-TTR trial⁴⁷, reduction in serum TTR reached steady-state levels by 13 weeks, with a mean reduction in serum TTR of 74% and median of 79%. Serious adverse events included glomerulonephritis (3%) and thrombocytopenia, with 3 patients having a platelet count < 25,000 (3%) and one death from intracranial hemorrhage (platelet count <10,000). These adverse effects have led to FDA approval of inotersen with a Risk Evaluation and Mitigation (REMS) Program that requires weekly monitoring of platelet counts and biweekly assessments of eGFR, urinalysis and urine creatinine protein ratio. Inotersen was being investigated in phase II programs for ATTR-CA, however, given the aforementioned side effects, toxicity profile and the development of longer acting ASOs, it is no longer being developed for use in ATTR-CA.

Patisiran (*Onpattro*, Alnylam), a siRNA which has been approved for ATTR-FAP in the US, targets the 3' untranslated region of the TTR mRNA. siRNA are double stranded, hydrophilic molecules containing a sense and an antisense strand and prone to rapid renal excretion⁴⁸. As such, patisiran is formulated as a lipid nanoparticle to target hepatocyte uptake. Once in the cytoplasm, the sense strand is removed by Ago2, an intracellular RNA endonuclease, leaving the pharmacologically active antisense strand-Ago2 complex to bind to the target mRNA. This forms the RNA-induced silencing complex (RISC) and facilitates subsequent degradation of the target mRNA. In a phase II study of patients with FAP, serum TTR levels were reduced by over 80% after the second dose of patisiran, when given at a dose of 0.3mg/kg every three weeks⁴⁹. The APOLLO study, a phase 3 study, randomized 225 patients with ATTR-FAP to patisiran vs placebo, excluding NYHA class III and IV patients⁵⁰. In the cardiac subpopulation (n=126) defined by a left ventricular wall thickness 13 mm at baseline and no history of hypertension or aortic valve disease, those who were randomized to patisiran demonstrated a reduction in mean LV wall thickness (~1mm), global longitudinal strain by -1.4% and N terminal pro-BNP (NT-proBNP) levels reduced by ~55%. A post-hoc analysis showed a reduction of 46% in cardiac hospitalizations and all-cause mortality. The APOLLO-B trial (NCT 03997383) is a phase III study of patisiran in 300 patients with ATTR-CA, with a 1:1 randomization to placebo. Subjects must be pre-medicated with antihistamines (H1 and H2), glucocorticoids and acetaminophen given the pro-inflammatory nature of the lipid nanoparticle-siRNA complex, which predominately manifests as infusion reactions. The study duration of APOLLO-B involves a 12-month double blind period followed by an open-label extension where all patients will receive treatment with patisiran. The primary endpoint will be change from baseline at month 12 in the 6-minute walk test.

Second Generation Therapies: Vutrisiran (Alnylam) is an siRNA which is conjugated to a N-acetyl galactosamine (GalNAc), specifically targeting hepatocytes. siRNAs that are conjugated to GalNAc enter cells via interaction with the GalNAc moiety on the asialoglycoprotein receptor (ASGPR) on the hepatocyte. ASGPR is present on the hepatocytes at a high concentration and thus facilitates rapid uptake. Vutrisiran has a

greater potency and longer duration of action than other knockdown therapies currently in clinical trial and as such can be administered at a lower dose (25 mg every 3 months), with lower injection volume and longer dosing intervals. Furthermore, as a subcutaneous injection it has greater ease of administration than IV infusions and no premedications are required given the absence of pro-inflammatory lipid nanoparticles in the formulation. In a phase 1 study ([NCT02797847](#)), a single dose of 25mg of vutrisiran resulted in a mean maximum TTR reduction of 83% by week 6, which was sustained for 90 days⁵¹, enabling quarterly administration. HELIOS-A was a phase III study which enrolled 164 patients with hereditary amyloidosis polyneuropathy with or without ATTR-CA, excluding NYHA class III-IV heart failure ([NCT03759379](#)). Patients received either vutrisiran or the reference comparator, patisiran, during the treatment period in a 3:1 randomization, after which all patients will be switched to vutrisiran during the treatment extension period. Vutrisiran met the primary endpoint, change in modified neurologic impairment score (mNIS+7) from baseline at month 9 as compared to historical placebo data from the APOLLO study in addition to both secondary endpoints (quality of life assessed by the Norfolk Quality of Life Questionnaire-Diabetic Neuropathy and gait speed assessed by the 10-meter walk test). In addition, vutrisiran showed improvement compared to placebo in the exploratory cardiac endpoint of change from baseline in NT-proBNP. HELIOS-B is a phase III study of approximately 600 ATTR-CA patients, randomized 1:1 to receive 25mg of vutrisiran every 3 months or placebo ([NCT04153149](#)). There will be a cap of 30% on those concurrently taking commercial tafamidis who are enrolled in the trial. The trial will run for 30–36 months with the primary outcome being a composite of all-cause mortality and recurrent CV hospitalizations.

AKCEA-TTR-LRx (ION 682884, Akcea Therapeutics, Inc) is a ligand (GalNAc linked to the 5' end) conjugated ASO (LICA) in which the ASO portion shares the same base sequence as inotersen. Thus, when the GalNAc is cleaved, ION-682884 has the same mechanism of action as inotersen, however the GalNAc conjugated drug has a much greater potency (approximately 51-fold). This allows for lower dose of drug to be administered to achieve a similar therapeutic effect. The ION-682884-CS1 ([NCT03728634](#)) study, showed a greater than 85% mean reduction in serum TTR levels with a monthly 45mg dose⁵². Furthermore, this dose (and interval of administration) has reportedly occurred without the problematic adverse events seen with inotersen, thought due to the 27-fold lower exposure to active drug seen with ION-682884. Cardio-TTRransform ([NCT04136171](#)) is a phase 3 clinical trial which will enroll approximately 700 patients with ATTR-CA, randomized 1:1 to receive 45mg of ION-682884 or placebo subcutaneously once every 4 weeks⁵³. All patients in this study will be allowed to receive commercial tafamidis concurrently. The treatment period will be 120 weeks with frequent clinical monitoring. The primary endpoint will be a composite of CV mortality and frequency of CV clinical events comparing the 2 study arms.

CRISPR /Cas9 stands for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 that is a genome editing approach which is being leveraged to knock down the production of hepatic transthyretin. Formulations in phase 1 human trials include NTLA-2001 that is composed of human single-guide ribonucleic acid and a messenger ribonucleic acid sequence encoding Cas9 protein encapsulated in a lipid

nanoparticle (LNP), that facilitates delivery to hepatocytes. The drug is a highly specific gene editing LNP that disrupts expression of human serum TTR expression. The first in human study is designed to investigate the safety and tolerability of NTLA-2001 at doses that are expected to meaningfully decrease the level of the circulating TTR protein. Animal data demonstrate that with a single administration, significant editing of the mouse transthyretin (TTR) gene in the liver was enabled with a >97% reduction in serum protein levels that persisted for at least 12 months⁵⁴.

Stabilizing the transthyretin tetramer (Table 3)

Diflunisal is a non-steroidal anti-inflammatory agent which binds to the T₄ binding sites on TTR, though with lower affinity than tafamidis. When compared to 3 other kinetic stabilizing agents, diflunisal was found to be the least potent. Dissociation of TTR was shown to be limited to 10% of normal with concentrations of 5.7 μM AG10 (800mg bid), 10.3 μM tolcapone (3 × 100mg over 12hours), 12.0 μM tafamidis (80mg daily) and 188 μM diflunisal (250mg bid)⁵⁵. Diflunisal is associated with adverse effects such as renal dysfunction, gastrointestinal bleeding, hypertension and fluid retention, which can exacerbate heart failure in susceptible individuals. However, when used in the treatment of ATTR-CA, diflunisal is prescribed at a dose of 250mg orally twice daily, which is below the dose recommended for anti-inflammatory activity and appears to be well tolerated⁵⁶. Furthermore, in subjects who receive this therapy, prescribers should ensure an eGFR > 45 ml/min, < 1 mg/kg of furosemide (or equivalent bioavailable loop diuretic) daily without a recent heart failure decompensation or history of gastrointestinal bleeding. Lohrmann et al assessed the effect of diflunisal therapy on cardiac structure and function at 1 year in 81 patients with ATTR-CA⁵⁷. They described a significant increase in serum TTR levels (33 vs 19 mg/dL, p=0.01), and improvement in both left atrial volume index (-1.4 vs +4.6 ml/m²) and cardiac troponin I (-0.01 vs +0.03 ng/ml, p=0.01) in treated compared with untreated groups. In addition, the ATTRwt subgroup were found to have stable global longitudinal strain on echocardiogram (+0.1% vs +1.2%, p=0.03 for treated vs untreated, respectively). As such, diflunisal has been used in an off-label manner in those without significant renal or hematologic comorbidities, and also in allele carriers of mutations who are at high risk of developing disease, with frequent monitoring of renal function and heart failure symptoms.

Tafamidis (Vyndamax/Vyndaqel, Pfizer Inc, New York, NY, USA)—In those of Portuguese descent the most common TTR variant is Val30Met (p.Val50Met), which leads to early onset familial amyloid polyneuropathy (FAP), often in the 4th decade of life. Val30Met (p.Val50Met) has a high clinical penetrance, though some carriers develop mild or no manifestations of disease. Coehlo and colleagues studied these individuals who were allele carriers of the Val30Met (p.Val50Met) variant but did not develop evidence of disease. They found that these individuals were compound heterozygotes for Val30Met/Thr119Met⁵⁸, which led to the hypothesis that a stabilizing variant (Thr119Met [p.Thr139Met]) can prevent dissociation of the TTR tetramer into monomers in the presence of a destabilizing variant and highly pathogenic Val30Met mutation. In seminal work, Kelly et al, showed that Thr119Met enhances stability of the TTR protein by slowing the rate of dissociation of the dimers³⁹. It was based on this discovery that the TTR stabilizer, tafamidis was developed. Tafamidis is a benzoxazole derivative which lacks nonsteroidal

anti-inflammatory activity and selectively binds to TTR in the blood, at the T₄ binding site. Bulawa et al, showed that tafamidis stabilizes wild type TTR and inhibits amyloidogenesis in a dose-dependent manner, with similar effects on both Val30Met and Val122Ile TTR variants. In a randomized clinical trial of 128 patients with Val30Met familial amyloid polyneuropathy (Fx-005), tafamidis 20mg daily delayed neuropathic progression and preserved quality of life compared with placebo in a predefined secondary analysis, after 18 months of treatment⁵⁹. It was approved by the European Medicines Agency in 2011 for patients with stage 1 symptomatic polyneuropathy. In the ATTR-ACT trial⁶⁰, 441 patients with ATTR-CA, NYHA class I-III HF were randomized to receive tafamidis 80mg, 20mg or placebo daily for 30 months. Tafamidis was associated with a lower all-cause mortality vs placebo (29.5% vs 42.9%) and a 32% lower risk of cardiovascular hospitalizations in those with NYHA class I or II HF. However, subjects with NYHA class III symptoms had higher rates of cardiovascular related hospitalization with tafamidis therapy compared to placebo, highlighting the importance of early diagnosis and treatment. Furthermore, assessment of functional capacity and quality of life parameters showed a lower rate of decline in the distance covered on 6-minute walk test and a lower rate of decline in the Kansas City Cardiomyopathy Questionnaire-Overall Summary score. It was based on this study that tafamidis became the first US Food and Drug Administration (FDA) approved TTR stabilizer to treat ATTR-CA in the US, in May 2019. It is formulated as tafamidis meglumine (20mg capsules, dose 80mg daily) and tafamidis free salt (61mg capsule daily), the latter of which was formulated for patient convenience as a single dose capsule. These formulations are bioequivalent, though are not substitutable on a per mg basis⁶¹.

AG10 (Eidos Therapeutics, Inc., San Francisco, CA, USA)—AG10 (*Acoramidis*) is a small molecule stabilizer with oral bioavailability which selectively binds to and stabilizes TTR. It mimics the super stabilizing properties of the Thr119Met variant⁶², which are thought to be due to the formation of hydrogen bonds between neighboring serine residues at position 117 of each monomer⁶³. AG10 has been shown to be a potent and selective stabilizer of TTR, exceeding the efficacy of tafamidis in stabilizing WT and variant TTR in serum⁶⁴. In the phase 2 AG10-201 study, there was near complete stabilization (>90%) of TTR at peak and trough serum levels, defined by percent occupancy of T₄ binding site as measured by Fluorescent Probe Exclusion⁶⁵. TTR stabilization was demonstrated by the fact that at 28 days after initiation of therapy, TTR levels rose on average by 51% in those taking 800mg twice daily of AG10 vs placebo. The Eidos AG10 study (ATTRIBUTE-CM) is a phase 3 trial, which has planned to enroll 510 subjects with ATTR-CA in a 2:1 ratio to AG10 800mg or placebo twice daily for 30 months (NCT03860935). The co-primary endpoints are change in distance walked on 6-minute walk test at 12 months (p<0.01) and all-cause mortality and frequency of cardiovascular related hospitalizations over a 30-month period (p<0.04). The 12-month data are expected towards the end of 2021.

Tolcapone—Tolcapone is a catechol-O-methyltransferase (COMT) inhibitor typically used for the treatment of Parkinson's disease, though also observed to have TTR stabilizing properties. *In vitro*, it was found that tolcapone has a high affinity for the T₄ binding site, displacing radiolabelled T₄ from TTR with 4 times the efficiency of tafamidis and stabilizing the TTR dimer-dimer interface. Furthermore, tolcapone exhibits stronger TTR aggregation

inhibitory activity for both wild-type and Val122Ile than tafamidis⁶⁶ demonstrating dose-dependent kinetic stabilization of the TTR tetramer. When studied *ex-vivo*, it was shown to bind to and kinetically stabilize tetrameric TTR in human plasma from subjects with wild-type TTR and carriers of Val30Met-TTR. However, tolcapone has a short half-life and a FDA black box warning of potentially fatal, acute liver failure.

With the advent of effective therapies for cardiac amyloidosis and the potential role of combination therapy, providers may soon be in the enviable position of choosing among therapies. The cost effectiveness of emerging therapies has been questioned^{67, 68}, especially for what may not be a rare disease (e.g. ATTRwt). Unfortunately, cost of such therapies could pose a significant obstacle to adoption and increase health disparities.

Anti-Plasma Cell Therapy (Table 4)

Therapies for AL amyloidosis have evolved considerably over the past decade. Autologous stem cell transplant (ASCT) has proven to have the best long-term outcomes, however only a fraction of patients with cardiac involvement are eligible and with a host of new, novel highly active therapies, the treatment landscape is rapidly evolving. The Mayo group reported that ~25% of patients are eligible for ASCT⁶⁹ and only 3.4% (23 of 668 patients) of patients with overt heart failure and AL amyloidosis treated at their center over a 20-year period underwent heart transplant⁷⁰. Cardiac damage in AL amyloidosis occurs as a result of light chain toxicity, and so the goal is rapid and complete normalization of the involved light chain. Indeed, the advent of effective anti-plasma cell therapies with limited toxicity has led to an evolution in the definition of a deep hematologic response from a complete response to modified, stringent and absolute involved free light chain response, the latter defined as a difference in involved and uninvolved light chain of < 10 mg/L⁷¹ and an involved free light chain of < 20 mg/L⁷². In those ineligible for ASCT, medical therapy is pursued with the goal of attaining complete remission (CR), defined as normalization of serum kappa and lambda free light chains and free light chain (FLC) ratio. Those who do not achieve CR may be classified as having very good partial response (VGPR), defined as a difference in FLC < 40mg/dl, partial response (PR, decrease in difference in FLC > 50%) or no response⁷³. Cardiac involvement of AL amyloidosis predicts a poor prognosis, which is outlined in the staging system created by the Mayo group. Patients are categorized as stage I to IV based on TnT 0.025 ng/ml (high sensitivity TnT 40ng/L), NT-proBNP 1800pg/ml and difference in FLC 18mg/dL, with stage I defined as all below threshold to stage IV being all three elevated³⁷. Typically, patients with a serum NT-proBNP 8,500 pg/ml or TnT 0.05 ng/ml (high sensitivity TnT 75 ng/mL) are considered ineligible for ASCT. Although patients may become transplant eligible over time, the benefit of ASCT compared to targeted anti-plasma cell therapy is unknown. Cardiac response is defined as a decrease in NT-proBNP by > 30% and 300 ng/L (if baseline NT-proBNP > 650ng/L)⁷³ and is associated with a survival benefit in the setting of anti-plasma cell therapy.

Alkylating Agents:

Melphalan, cyclophosphamide and bendamustine belong to a group of chemotherapeutic agents called the nitrogen mustard alkylating agents. Melphalan exerts its effects via

alteration of the DNA nucleotide guanine, and results in the formation of inter- and intra-strand DNA crosslinks⁷⁴ which interfere with DNA replication and transcription. The cytotoxic effects of melphalan are related to cellular concentration and duration of cellular exposure, with some studies linking higher doses of melphalan to excess toxicity in older patients and those with renal impairment. As such, a lower dose of melphalan (~140 mg/m²) is often used in those over 65 years, and in patients with renal insufficiency, reduced cardiac function or lower performance status without a detrimental effect on long-term outcomes⁷⁵. Oral melphalan is typically combined with dexamethasone in the treatment of patients who are ineligible for ASCT or as pre-ASCT conditioning treatment. Palladini et al evaluated 259 patients with AL amyloidosis treated with oral melphalan and dexamethasone, over 50% of whom had advanced cardiac disease⁷⁶. In those with severe cardiac involvement, the dose of dexamethasone was reduced by 50% given the propensity for fluid retention and exacerbation of heart failure. Hematologic response rate in the full dose group was 76% vs 51% in the attenuated steroid dose group with a complete response occurring in 31% and 12% respectively. Median survival was also much lower in the attenuated dose group, 20 months as compared to 7.4 years in the full dose group. Patients with severe cardiac involvement are at high risk of early death after the initiation of treatment, have a short overall median survival of < 18 months and poorer response to therapy⁷⁷. Cyclophosphamide acts in a similar manner to melphalan, though exerts its effect via the active metabolite, phosphoramidate mustard, at the guanine N-7 position leading to cell apoptosis⁷⁸. It is typically used in combination with other therapies such as proteasome inhibitors and CD38 monoclonal antibodies and is associated with side effects including hair loss and hemorrhagic cystitis. Bendamustine was investigated in a phase 2 trial of 31 patients with relapsed AL amyloidosis in combination with dexamethasone, in which 57% achieved a partial response or better (NCT01222260)⁷⁹. The overall organ response was 29% with a median overall survival of 18.2 months. Bendamustine is considered a third line or salvage therapy.

Steroids:

Glucocorticoids (prednisone and dexamethasone) bind to cytosolic glucocorticoid receptors and translocate to the nucleus where they modulate gene expression resulting in anti-inflammatory and immunosuppressive activity⁸⁰. Dexamethasone treatment induces upregulation of pro-apoptotic genes, down-regulation of anti-apoptotic genes and activation of intrinsic apoptotic pathways⁸⁰. It has a potency 6-fold that of prednisone, however, with greater efficacy comes greater toxicity, particularly in patients with advanced cardiac involvement. In a study by Dhopaker et al, 28% of patients experienced volume overload related to treatment with 40mg daily of dexamethasone (480mg per cycle)⁸¹. Treatment related mortality occurred in 6 patients, 4 of whom had advanced cardiac amyloidosis and experienced sudden death thought due to a cardiac event. Other severe adverse effects include thrombosis, gastrointestinal hemorrhage, infections and psychosis especially with high dose therapy and as such a low-dose strategy is often employed in older patients and those with heart failure⁷⁶. In the recent era, due to their synergistic effect with immunomodulatory agents and proteasome inhibitors, glucocorticoids are used as part of a multidrug regimen to improve response rates and limit toxicity (see below).

Immunomodulatory agents

Originally marketed as a tranquilizer and antiemetic, thalidomide and its derivatives (lenalidomide and pomalidomide) have antiproliferative, anti-angiogenic and immunomodulatory effects. Their anti-angiogenic and antiproliferative properties are mediated via inhibition of interleukin-6 (IL-6) expression, a growth factor for the proliferation of myeloma cells. They activate apoptotic pathways through direct activation of caspase-8 mediated cell death. Within the mitochondria, they activate c-jun terminal kinase (JNK) which through a series of events result in the release of pro-apoptotic proteins into the cytosol⁸². In addition, by activating T cells, they increase expression of IL-2 and interferon-gamma (IFN- γ) genes, augmenting natural killer (NK) cell-dependent cytotoxicity⁸². Lenalidomide has a greater potency than thalidomide, being 50 to 2,000 times more potent at stimulating T-cell proliferation and 50 to 100 times more potent in augmenting IL-2 and IFN- γ production⁸². Dexamethasone activates caspase-9, a pro-apoptotic molecule and is associated with the release of second mitochondrial-derived activator of caspase (Smac) from the mitochondria into the cytosol, a regulator of the activity of molecules that affect apoptosis⁸². As such, it augments the anti-proliferative effects of lenalidomide. However, notable side-effects include neutropenia, thrombocytopenia, elevations in liver function tests and thromboembolism and lenalidomide tends to raise NT-proBNP in AL amyloidosis patients⁸³. With the addition of melphalan to lenalidomide and dexamethasone, overall hematologic response rates of ~ 58% are observed⁸⁴, with a higher dose of lenalidomide associated with a higher rate of CR, though limited by tolerability at doses >15mg⁸⁴. The addition of cyclophosphamide in place of melphalan shows similar overall hematologic response rates (~60%), though again has a high burden of toxicity and poor outcomes in those with advanced cardiac disease⁸⁵. Overall hematologic response rates with pomalidomide/dexamethasone are similar to those achieved with lenalidomide (40–60%)^{85, 86}. Unfortunately, in those with advanced cardiac amyloidosis at the time of treatment initiation, outcomes of these treatment combinations remain poor.

Proteasome Inhibitors:

The ubiquitin-proteasome system (UPS) functions as a pathway for intracellular regulation of protein degradation, thus playing a role in maintaining protein homeostasis⁸⁷. The UPS involves a series of enzymes which tag the protein with a polyubiquitin chain. The 26S proteasome comprises a 20S core flanked by two 19S caps. The 20S core contains subunits which have proteolytic activity, including caspase-like, trypsin-like and chymotrypsin-like activity. The proteasome recognizes and binds the tagged protein and subsequently hydrolyzes it into short polypeptides in the 20S core. Proteasome inhibitors bind to the proteasome binding pocket, thus rendering it inactive⁸⁷ which results in a multitude of downstream events, including accumulation of ubiquitin tagged proteins, inhibition of NF- κ B signaling, downregulation of growth factor receptors, suppression of adhesion molecule expression and inhibition of angiogenesis, all of which lead to apoptosis⁸⁸. Bortezomib (Velcade) is a first-generation reversible boronic acid inhibitor of the chymotrypsin-like activity to the proteasome. Bortezomib, in conjunction with cyclophosphamide and dexamethasone (CyBorD), has been shown to induce a rapid reduction in light chains in patients with AL amyloidosis and appears to be relatively well tolerated in those with cardiac involvement, however, can cause significant peripheral neuropathic side effects.

Furthermore, those with translocation t(11;14) have inferior hematologic response to bortezomib⁸⁹. A study of 230 patients from the National Amyloidosis Center (London, England) and the Amyloidosis Research and Treatment Center (Pavia, Italy) of newly diagnosed patients with AL amyloidosis reported an overall hematologic response rate of 60% with a cardiac response of 17%⁹⁰. Advanced cardiac stage IIIb patients (Mayo 2004 criteria⁹¹) (NT-proBNP > 8500ng/L) had a lower overall response of 42% and poorer survival (median survival 7 months), than those with stage II or IIIa disease (overall hematologic response 64% and 69%, respectively), though still showed a survival benefit in those who achieved a hematologic response compared to those who did not (median survival 26 months vs 6 months, respectively)⁹⁰. In a retrospective study of bortezomib/melphalan/dexamethasone (BMDex) vs melphalan/dexamethasone (MDex), the former induced higher hematologic response (69 vs 51%) and CR (42 vs 19%) the difference in response between groups being most notable in those who could not tolerate full-dose dexamethasone⁹². The addition of bortezomib in patients with NYHA class III or IV HF and/or NT-proBNP > 8500ng/L did not improve survival. Second generation proteasome inhibitors include carfilzomib, ixazomib, marizomib and oprozomib. Carfilzomib (Kyprolis) is an irreversible tetrapeptide epoxyketone which has greater inhibitory activity than bortezomib and has shown activity in patients resistant to bortezomib⁹³. In a phase 1 dose-escalation study of carfilzomib in patients with previously treated systemic AL amyloidosis however, cardiac events were common⁴². Three of 12 patients had a cardiac event: 1 with cardiac arrest due to ventricular tachycardia, 1 developed a restrictive cardiomyopathy (amyloid negative on biopsy) and 1 had a decline in LV function, all possibly related to carfilzomib therapy and highlighting the need for close cardiac monitoring if this therapy is utilized. However, in those for whom bortezomib is contraindicated due to peripheral neuropathy, carfilzomib may be effective in appropriately selected candidates without severe cardiac involvement⁹⁴. Ixazomib (Ninlaro) is a reversible agent which is hydrolyzed to its active form in aqueous solution and has comparable inhibitory activity to bortezomib⁹³. It binds to the proteasome beta-5 site to inhibit the chymotrypsin-like activity. In 27 patients with relapsed/refractory AL amyloidosis ixazomib showed encouraging hematologic (52%) and organ (56%) response rates with a 45% cardiac response rate (5 of 11 patients)⁹⁵. It is being investigated in a phase 1/2 study to assess safety and hematologic response rate in combination with cyclophosphamide and dexamethasone (NCT03236792). Patients with NYHA class III or IV HF or NT-proBNP > 8500ng/L are excluded. TOURMALINE-AL1 is a phase 3 trial investigating the use of ixazomib with dexamethasone vs physician's choice (NCT01659658), results of which showed no significant difference in hematologic overall response rate⁹⁶. Nevertheless, CR was more frequent with ixazomib (26 vs 18%), and treatment with ixazomib was associated with a significantly longer progression-free survival. Furthermore, the patients who received ixazomib had a higher rate of cardiac and renal responses.

Daratumumab:

CD38 is a glycoprotein expressed on a variety of cell types, including normal myeloid and lymphoid cells, but is also highly overexpressed on neoplastic monoclonal plasma cells⁹⁷. CD38 is involved in cell signaling and regulation of cytoplasmic calcium flux playing a role in activation, survival and growth of lymphoid and myeloid cells. Daratumumab (Darzalex)

is a human immunoglobulin G1 kappa (IgG1 κ) monoclonal antibody against CD38 antigen which leads to cell death via multiple mechanisms including complement mediated and antibody dependent cytotoxicity and apoptosis⁹⁸. It has been shown to be highly effective in and is approved for the treatment of multiple myeloma⁹⁹, which led to investigation of its use in AL amyloidosis. It has been shown to be effective in the treatment of patients with relapse or progression of AL amyloidosis, resulting in a 76% hematologic response rate and 36% CR rate with a median time to response of 1 month¹⁰⁰. The speed with which daratumumab can achieve normalization of light chain levels was highlighted in a report by Hossein et al¹⁰¹. Two patients with advanced Mayo cardiac stage (stage III and IV) were treated with daratumumab monotherapy and achieved a normal light chain level within one cycle of therapy. ANDROMEDA investigated the safety and efficacy of daratumumab plus CyBorD compared with CyBorD alone in patients with new diagnosed AL amyloidosis (NCT03201965). ANDROMEDA enrolled 388 patients, randomized to CyBorD alone or with daratumumab (1:1). More than one-half of patients assigned to daratumumab achieved a complete hematological response (53%) compared with only 18% of patients assigned to CyBorD (odds ratio [OR] = 5.1; p<0.0001). The 6-month cardiac response rate was 42% for the daratumumab arm compared with 22% for CyBorD alone (p=0.0029)¹⁰². In comparison, CR rates in patients receiving CyBorD⁹⁰, melphalan with dexamethasone⁷⁶ or HDM with ASCT¹⁰³ have been reported as 23%, 19% and up to 48%, respectively. Isatuximab acts by inducing internalization of CD38¹⁰⁴. Daratumumab was approved by the FDA in combination with CyBorD for newly diagnosed light chain amyloidosis. A phase 2 study of isatuximab in patients with relapsed or refractory AL amyloidosis with organ involvement is underway (NCT03499808), however those with NYHA class IV symptoms or EF < 35% are excluded from participation. Elotuzumab is an IgG1 κ monoclonal antibody against signaling lymphocytic activation molecule F7 (SLAMF7) which is used in the treatment of multiple myeloma combined with lenalidomide and dexamethasone. The mechanism of action in multiple myeloma cells appears to be antibody-dependent, cell-mediated cytotoxicity through recruitment and activation of NK cells¹⁰⁵. Elotuzumab mediated cell death requires the presence of NK cells since binding to SLAMF7 marks the myeloma cells for recognition by NK cells and also direct binding to SLAMF7 on NK cells themselves causes direct activation and enhanced cytotoxicity¹⁰⁶. Elotuzumab in conjunction with lenalidomide and dexamethasone was successful in inducing hematologic and renal response in a patient with relapsed/refractory multiple myeloma with AL amyloidosis despite two stem cell transplants and numerous combinations of chemotherapeutic agents¹⁰⁷. It is currently being studied in a phase 2 trial in conjunction with lenalidomide and dexamethasone with or without cyclophosphamide in patients with relapsed AL amyloidosis (NCT03252600).

Venetoclax

Translocation t(11;14) is the most common cytogenic aberration in AL amyloidosis occurring in up to 60% of patients and confers a poor response to bortezomib⁸⁹. Patients with t(11;14) overexpress B-cell lymphoma 2 (BCL-2), a protein involved in programmed cell death regulation¹⁰⁸. BCL-2 mediates suppression of the proapoptotic pathway molecules BAX and BAK¹⁰⁸. Venetoclax, an orally bioavailable agent, is an inhibitor of BCL-2 and thus promotes cell death. It has been shown to be effective in patients with multiple myeloma with translocation t(11;14)¹⁰⁹. A phase 1 study (NCT03000660) investigating

venetoclax and dexamethasone in relapsed/refractory AL amyloidosis was stopped given the FDA concerns that emerged from the BELLINI clinical trial ([NCT02755597](#), Study M14–031) evaluating venetoclax with bortezomib in patients with multiple myeloma in which there was an increased risk of death for patients receiving venetoclax as compared to the control group. However, after subgroup analysis, the BELLINI study has reopened for patients with t(11;14). Similarly, it is expected that the study of venetoclax in AL amyloidosis will reopen for those with t(11;14).

Amyloid Degradation/Extraction:

Doxycycline (Table 3) is a tetracycline antibiotic which binds to the bacterial ribosome and inhibits protein synthesis. However, it is the ability to inhibit matrix metalloproteinase (MMP) which is thought to be the basis of its anti-amyloidogenic activity¹¹⁰. Levels of MMP are elevated in AL accompanied with marked diastolic dysfunction when compared to little or no elevation in TTR amyloidosis¹¹⁰. This led to a phase 2 study of the safety and efficacy of doxycycline in combination with CyBorD for the treatment of AL amyloidosis. D'Souza and colleagues reported a low 1-year mortality of 20% and a high stem cell transplant utilization rate of 60%, in this single arm study¹¹¹.

The use of doxycycline in combination with ursodeoxycholic acid (ursodiol) for the treatment of ATTR CA was studied in 53 patients¹¹². Ursodiol is a bile acid sequestrant which has anti-amyloid fibril effects as has its taurine conjugate, tauroursodeoxycholic acid (TUDCA). Both have synergistic activity with doxycycline to reduce amyloid fibril burden. Karlstedt et al reported an 11% adverse event rate, mainly due to dermatologic and gastrointestinal side effects, and equivocal outcomes¹¹² with no obvious benefit observed. Overall, it remains unclear as to the place doxycycline and ursodiol have in the treatment of either AL- or ATTR-CA.

NI006 is a recombinant human monoclonal IgG1 antibody that exclusively targets with high affinity the forms of TTR that are disease-associated with amyloid conformation but not physiological forms of transthyretin. NI006 targets both wild-type TTR as well as TTR variants and induces the clearance of pathological TTR in preclinical models. Currently, NI006 is in Phase 1 clinical development in ATTR cardiomyopathy patients ([NCT04360434](#)).

CAEL-101 (11–1F4) is an IgG1 monoclonal antibody that binds to kappa and lambda light chain amyloid fibrils, accumulating in amyloid laden organs¹¹³ leading to elimination of the amyloid protein. In the 1 year follow up of the phase 1a/1b study, 67% (12 of 18) of renal and/or cardiac-evaluable patients demonstrated organ response⁴³. Currently, CAEL-101 is being evaluated in patients with Mayo stage IIIa ([NCT04512235](#)) and IIIb cardiac AL amyloidosis ([NCT04504825](#)).

Serum amyloid P (SAP) is a glycoprotein which binds avidly to all types of amyloid fibrils. As such, targeting this protein may provide a pathway to extraction of amyloid deposits from affected organs. Bodin et al showed that administration of anti-human-SAP antibodies to mice with amyloid deposits containing human SAP activated macrophage mediated phagocytosis of the SAP containing amyloid deposits¹¹⁴.

In a phase 1 trial, a small-molecule drug, (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC), was first administered to 15 patients with systemic amyloidosis to reduce circulating SAP, followed by a fully humanized monoclonal IgG1 anti-SAP antibody⁴¹. Patients with cardiac involvement were excluded from the study. At 42 days following treatment, there was a decrease in amyloid organ burden as indicated by decreased hepatic stiffness and reduction in hepatic amyloid burden on SAP scintigraphy. A phase 2 study failed to show any improvement in cardiac amyloid burden after treatment with anti-SAP antibody (dezamizumab).

NEOD-001 (Prothena) is a humanized form of murine monoclonal antibody 2A4, which binds to an epitope on the misfolded light chain protein though does not bind in the protein's native conformation. Therefore, it was posited that NEOD-001 could clear AL amyloid deposits in affected organs. Exploratory end points of a phase 1/2 study showed that 8 of 14 patients (57%) with cardiac involvement responded to therapy while the remaining 6 patients showed stable disease¹¹⁵. A phase 2b study (NCT02632786) in previously treated patients with persistent cardiac dysfunction did not meet its primary or secondary endpoints and the phase 3 study in treatment naïve patients was discontinued due to futility.

PRX004 (Prothena) is a monoclonal antibody which selectively binds to non-native misfolded TTR but not native TTR. PRX004 is being studied in patients with hereditary ATTR amyloidosis in a phase 1 study (NCT03336580).

Emerging therapeutics:

Anti-seeding (Table 3) refers to inhibition of the aggregation of native transthyretin onto preformed amyloid fibrils. This may be particularly beneficial in the setting of single organ liver transplantation for ATTRv, after which ongoing cardiac deposition can occur. Preformed amyloid fibrils in the heart can act as a template for further seeding of native transthyretin. TabFH2 is a peptide inhibitor which binds to the amyloid driving F- and H-stands of fragmented fibrils, thereby impeding self-recognition and seeding⁴⁵.

The CD38-targeting antibody-drug conjugate (ADC) STI-6129/CD38-077, comprises a fully human anti-CD38 antibody conjugated to a microtubule inhibitor (duostatin 5.2)¹¹⁶. This ADC binds avidly to CD38 positive tumor cells, after which it is internalized to exert its cytotoxic effect. STI-6129 is being studied in patient with relapsed or refractory systemic AL amyloidosis in a phase 1 study (NCT04316442).

Advanced Therapies

Heart transplantation may be considered in select patients with end-stage cardiomyopathy secondary to ATTR-CA or AL-CA who have responded to light-chain depleting therapy. Heart transplant candidates should be carefully selected with particular attention being paid to extracardiac involvement such as neuropathic, gastrointestinal or hepatic manifestations of disease, which could affect post-transplant outcomes¹¹⁷. Patients with ATTRwt- or ATTRv-CA with the Val122Ile mutation generally require only single organ heart transplant, however, other variants, such as Thr60Ala may need consideration for dual heart/liver transplant. Recent single center studies have shown that outcomes of appropriately selected

patients with either AL- or ATTR-CA are similar to that of non-amyloid cardiomyopathy patients^{118, 119}. Furthermore, the new heart transplantation allocation policy¹²⁰ provides a pathway for listing these patients with a restrictive cardiomyopathy, who often do not meet the hemodynamic criteria otherwise required for higher priority status. This has led to a decrease in waitlist time/delisting due to clinical deterioration and an increase in the number of patients being transplanted with amyloid cardiomyopathy¹²¹ without any change in short-term outcomes. Going forward, it is unclear where heart transplantation will fit in the treatment paradigm, as earlier detection of amyloid cardiomyopathies and novel medical therapies continue to change the landscape of this disease.

Summary/Conclusions

Basic science investigations in the last few decades have led to the elucidation of mechanisms underlying amyloidogenesis and have resulted in the development of effective therapies in multiple classes of compounds. The cardiologist caring for affected patients is in the enviable position of choosing from these therapies that can meaningfully improve the lives of affected patients especially when administered before significant cardiac dysfunction has ensued (Figure 4). The therapeutic landscape in this arena is rapidly evolving and additional breakthroughs are anticipated in the coming years.

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Non-standard Abbreviations and Acronyms

AL	immunoglobulin light chain
ASCT	autologous stem cell transplant
ASGPR	asialoglycoprotein receptor (ASGPR)
ASO	antisense oligonucleotide
ATTRv	variant transthyretin amyloidosis
ATTRwt	wild-type transthyretin amyloidosis
BCL-2	B-cell lymphoma 2 receptor
BMDex	bortezomib, melphalan, dexamethasone
BNP	brain natriuretic peptide
CA	cardiac amyloidosis
COMT	catechol-O-methyltransferase

CPHPC	(R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid
CR	complete response
CRISPR/Cas9	clustered regularly interspaced short palindromic repeats/ CRISPR-associated protein 9
CyBorD	cyclophosphamide, bortezomib, dexamethasone
FAP	familial amyloid polyneuropathy
GalNAc	N-acetyl galactosamine
HDM	high dose melphalan
IAA	isolated atrial amyloidosis
IFN-γ	interferon-gamma
JNK	c-jun terminal kinase
LICA	ligand conjugated antisense oligonucleotide
LNP	lipid nanoparticle
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
NT-proBNP	N terminal pro-BNP
PR	partial response
SAP	serum amyloid P-component
Smac	second mitochondrial-derived activator of caspase
siRNA	small interfering ribonucleic acid
SLAMF7	signaling lymphocytic activation molecule F7
SSA	Serum amyloid associated protein
Tn	troponin
TUDCA	tauroursodeoxycholic acid
UPS	ubiquitin-proteasome system
VGPR	very good partial response

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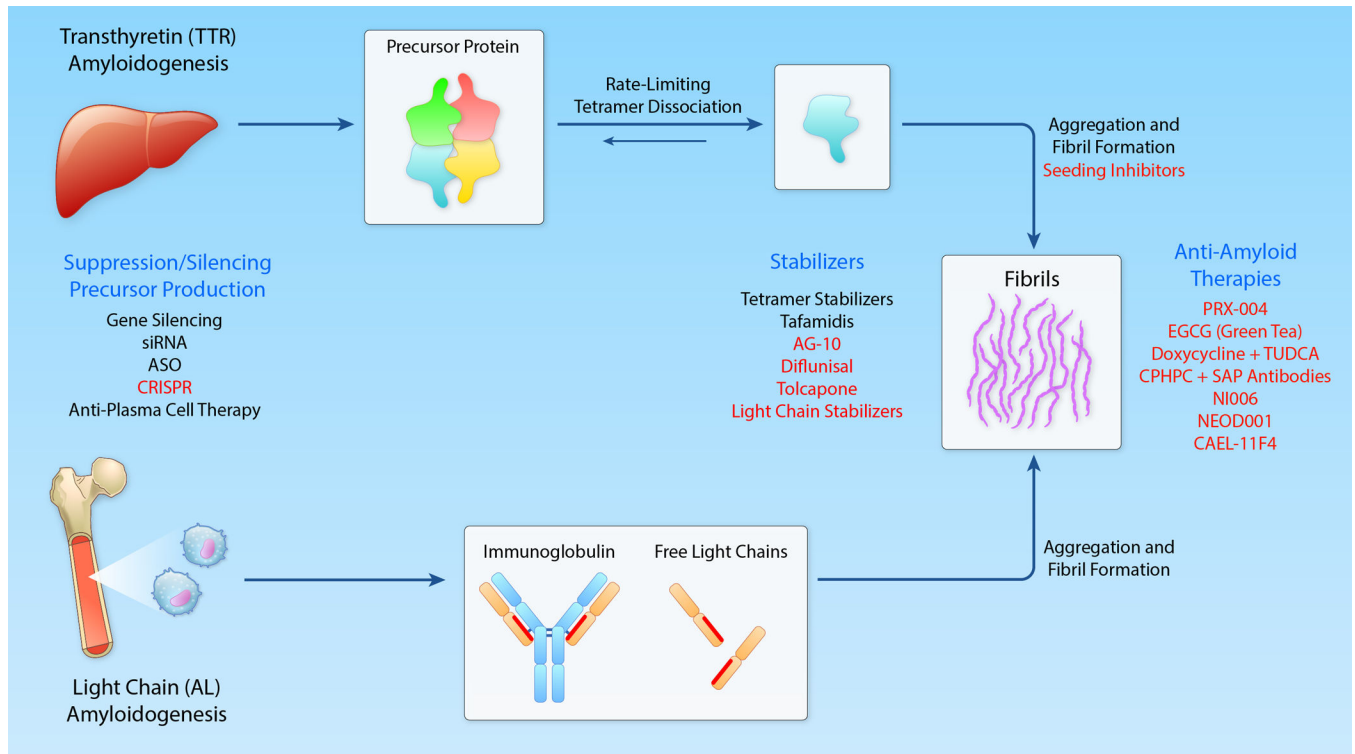


Figure 1: Pathogenesis of Cardiac Amyloidosis and Therapies. Mechanisms underlying formation of cardiac amyloidosis in TTR and AL. Targets for therapy are enumerated in blue. Effective therapies approved for use are shown in black and experimental therapies in a particular class are shown in red. Those listed above specific mechanism are for TTR while those listed below a specific mechanism are for AL amyloidosis. (Illustration credit: Ben Smith).

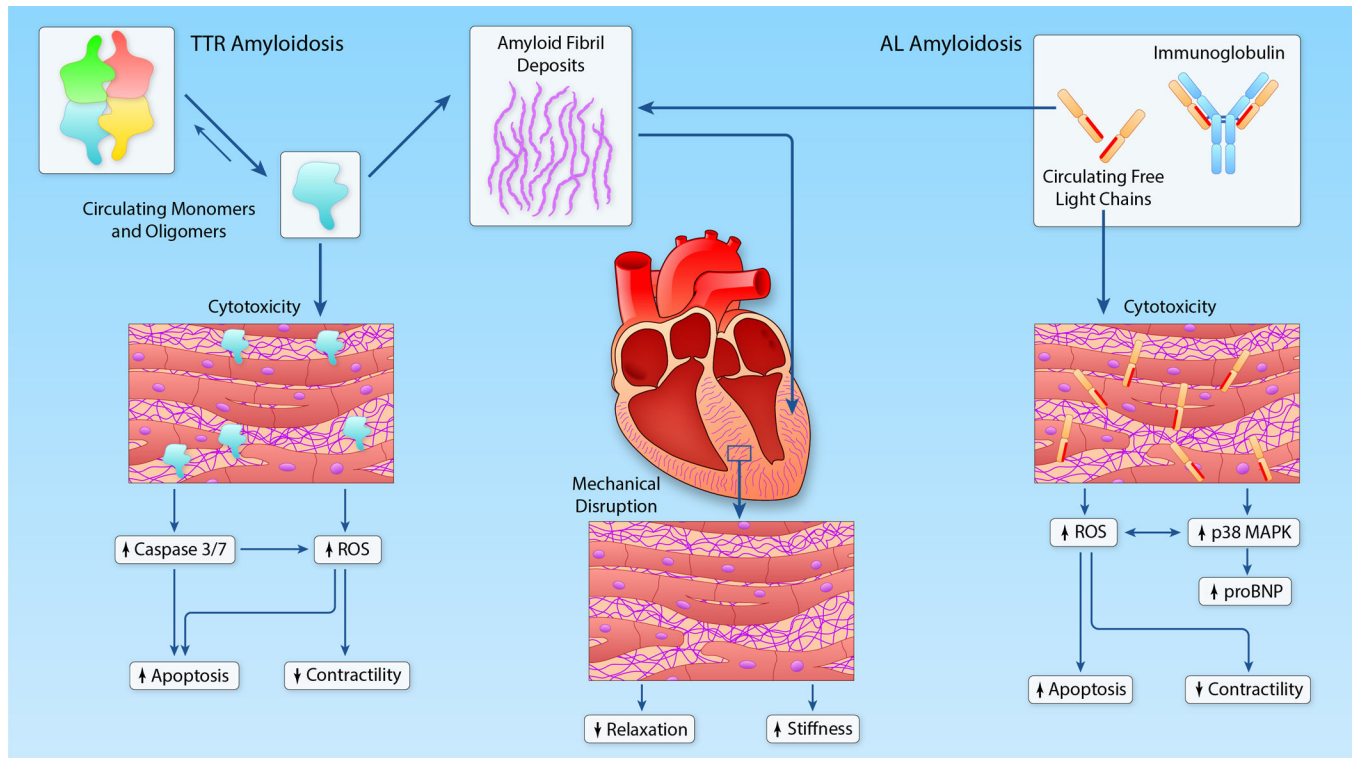


Figure 2: Mechanisms of myocardial dysfunction in TTR and AL amyloidosis. In both TTR and AL, extracellular deposition of amyloid fibrils causes mechanical disruption of normal tissue architecture, leading to impaired relaxation and increased ventricular stiffness. In AL amyloidosis, circulating free light chains cause cytotoxicity by increasing oxidative stress and activation of the p38 MAPK signaling pathway. Activation of the p38 MAPK pathway also leads to release of NT-proBNP. In TTR amyloidosis, circulating TTR monomers and oligomers have also been proposed to cause direct cytotoxicity. (Illustration credit: Ben Smith).

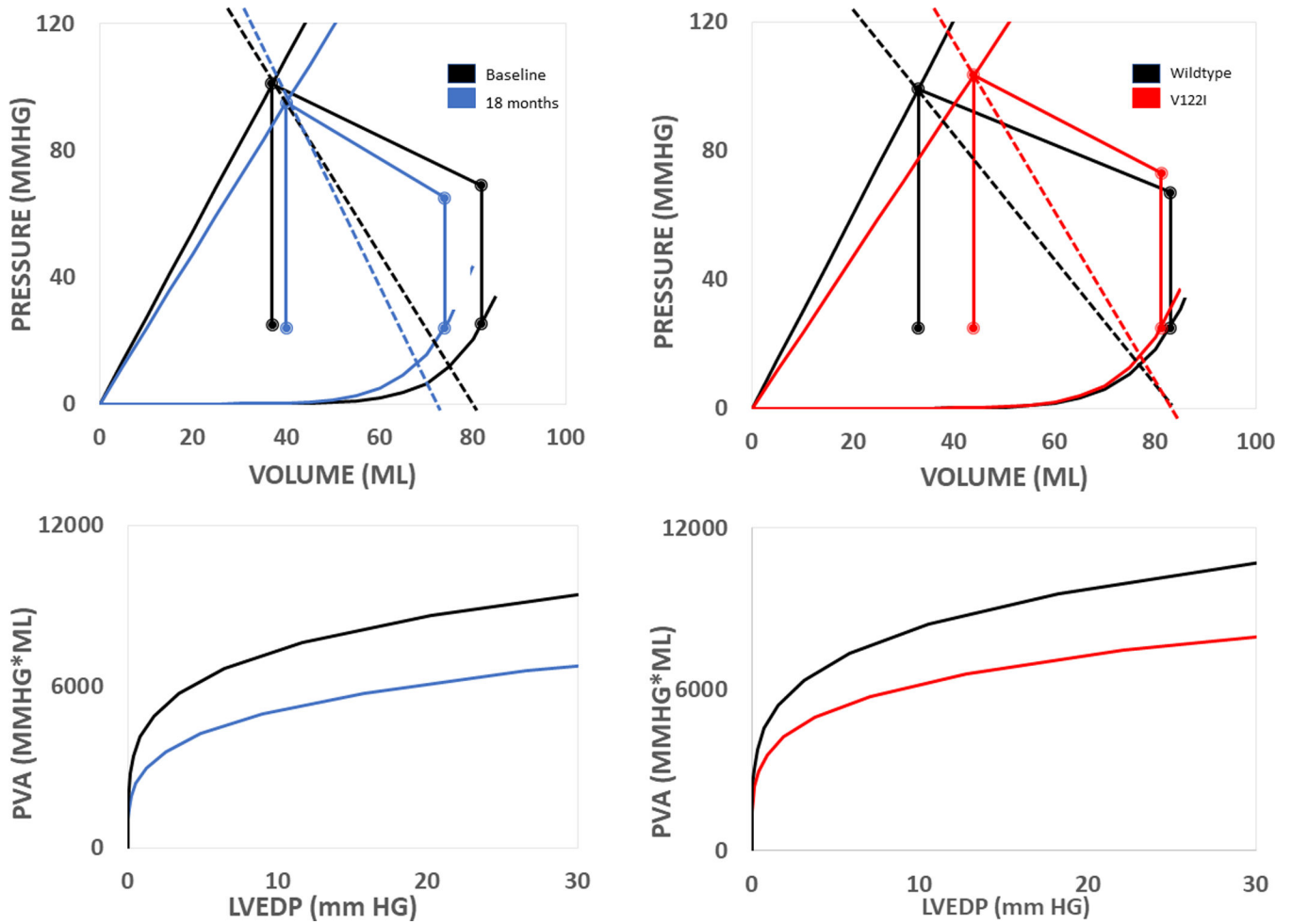


Figure 3:

Cardiac mechanics in TTR amyloidosis. Non-invasive pressure-volume loops (top) and isovolumetric pressure volume area (bottom).

(Left) Progression of cardiac chamber dysfunction overtime marked by reduced ventricular capacitance and impaired contractility. Collectively, both abnormalities lead to reduced stroke volume and isovolumetric pressure volume area overtime.

(Right) Compared to WT, patients with V122I associated ATTR-CA have more impaired cardiac function at baseline. Patients with both WT and V122I associated ATTR-CA have reduced ventricular capacitance. However, patients with V122I associated ATTR-CA also have impaired contractility, leading to lower stroke volume and isovolumetric pressure-volume area in V122I associated ATTR-CA compared to WT.

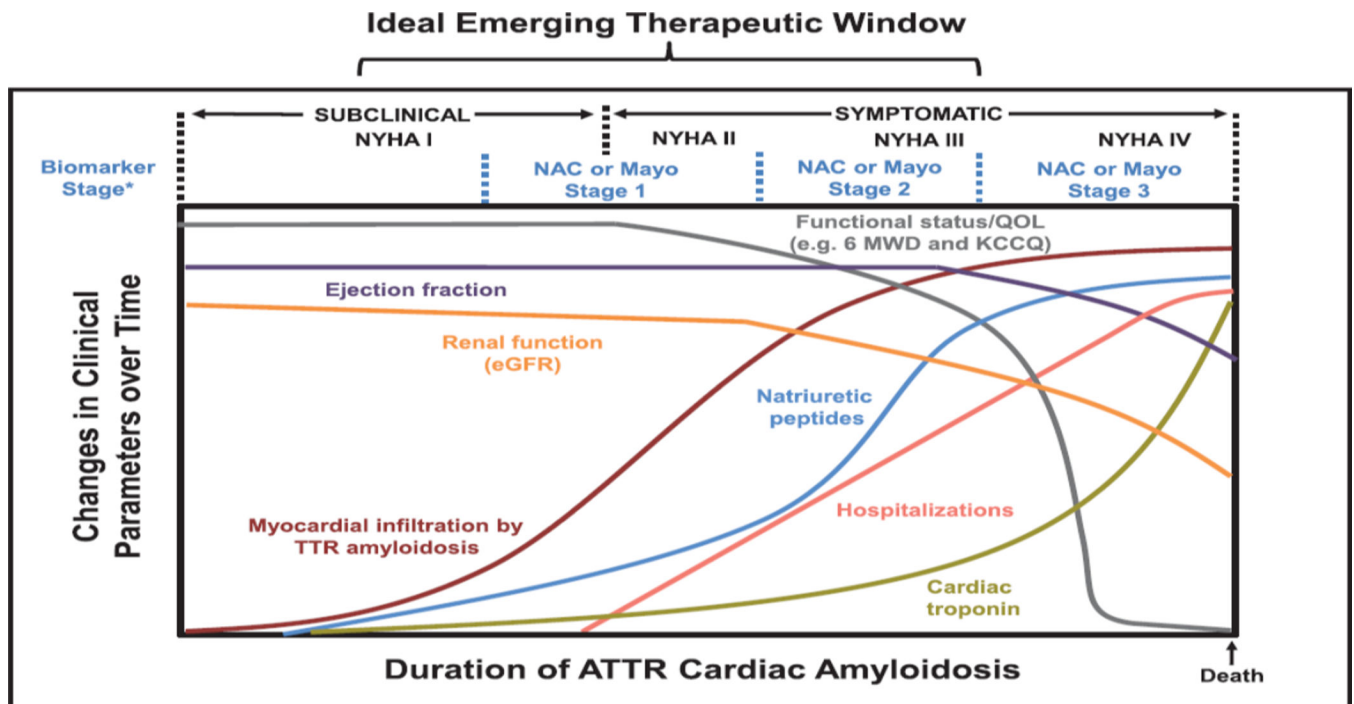


Figure 4:

Model of ATTR-CA progression over time. Myocardial amyloid infiltration occurs before clinically manifested changes in ejection fraction, cardiac biomarkers, and renal function. The ideal emerging therapeutic window for novel therapies is hypothesized to be before significant organ dysfunction has occurred and before rapid and potentially irreversible declines in functional capacity. The relative scale specific to each factor and time course are not proportional. (From *Circulation*. 2019 Jul 2;140(1):27–30).

Table 1.

Major Etiologies of Cardiac Amyloidosis

Features	Light Chain Cardiac Amyloidosis (AL-CA)	Transthyretin Cardiac Amyloidosis	
		Wild type (ATTRwt-CA)	Variant / Hereditary Transthyretin (ATTRv-CA)
Age at diagnosis	5th to 9th decade	7th to 10th decade	3rd to 8th decade
Sex distribution	Roughly equal male:female	Very significant male predominance	Male predominance
Precursor protein	Light-chain	Transthyretin	Transthyretin
Genetic etiology	None	None	Autosomal dominant inheritance
Genetic modifier to therapeutic efficacy	t(11,14) presence – poor response to bortezomib but responsive to venetoclax	None	None
Extracardiac involvement	Nerves, kidney, liver, gastrointestinal tract, skin, tongue/soft tissue	Carpal tunnel, lumbar spine, gastrointestinal tract	Nerves,
Clinical Manifestations	Multi-systemic disease with cardiac and renal involvement (60–70%); liver (15%) and peripheral / autonomic neuropathy (10%)	Predominant cardiac phenotype with a restrictive cardiomyopathy, atrial and ventricular arrhythmias and HFpEF	Depends on variant. Val122Ile predominately cardiac, Thr60Ala mixed and Val30Met predominately neuropathic
Prognosis after diagnosis	Depends on stage. Median survival 4–6 months with advanced heart failure	Depends on stage. Median survival 2–6 years in the absence of treatment	Depends on mutation and stage. Median survival 3–12 years

AL-CA, immunoglobulin light-chain cardiac amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; ATTRv, variant (hereditary, familial) transthyretin amyloidosis; CA, cardiac amyloidosis; HFpEF, heart failure with a preserved ejection fraction.

Table 2:

Clinical manifestations and diagnostic evaluation of cardiac amyloidosis

	AL (Light Chain Amyloidosis)	TTR (Transthyretin Amyloidosis)
Clinical features	Heart failure with a preserved ejection fraction Right heart failure (JVP, hepatomegaly and edema) Atrial arrhythmias (atrial fibrillation and flutter) Intolerance of neurohormonal blockade	
	Periorbital purpura and macroglossia Proteinuria and nephrotic syndrome Peripheral neuropathy or autonomic dysfunction Gastrointestinal motility disorder	Men affected more than females in ATTRwt Peripheral neuropathy or autonomic dysfunction especially in ATTRv Orthopedic manifestations including bilateral carpal tunnel, lumbar spinal stenosis and biceps tendon rupture Conduction disease
ECG	Low voltage to mass ratio Pseudoinfarct pattern Low QRS voltage (late phase phenomenon, not sensitive but specific)	
	Low voltage more common due to toxic light chains	Conduction disease Left Ventricular Hypertrophy in 15%
Echocardiogram	Left ventricular wall thickness >12mm Low tissue doppler velocities Restrictive filling pattern Apical sparing Atrial septal thickening	
	LV wall thickness usually < 15 mm	LV wall thickness usually > 15 mm Concomitant aortic stenosis
Cardiac Magnetic Resonance Imaging	Late gadolinium enhancement (in any pattern) Elevated native T1 and ECV	
	ECV higher in AL than ATTR	Native T1 lower in ATTR than AL
^{99m}Techetium-pyrophosphate scintigraphy	Myocardial uptake < grade 2	Myocardial uptake grade 2
Endomyocardial or extracardiac biopsy	Positive congo red staining Apple-green birefringence under polarized light	
	Fat pad biopsy positive > 50% Renal involvement Carpal tunnel	Fat pad biopsy positive < 50% Lumbar spine and carpal tunnel
Serum Biomarkers	Elevated troponin and natriuretic peptides based on stage of disease	
	Abnormal free light chain ratio Monoclonal protein present on immunofixation	Normal free light chain ratio No monoclonal protein on immunofixation

AL, immunoglobulin light-chain amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; ATTRv, variant transthyretin amyloidosis; ECV, extracellular volume; JVP, jugular venous pressure; LV, left ventricle.

Table 3:

Therapies for Transthyretin Cardiac Amyloidosis

	Drug	Phase of study	Indication by Amyloid Type	Mechanism of action	Dose	Adverse Effects	Concomitant Therapy & Monitoring	Annual Cost	Primary Endpoints
KNOCK-DOWN	Inotersen (<i>Tegsedi</i>)	Phase 2 NCT03702829	ATTRv & ATTRwt-CA NYHA I-III	2'-O-methoxyethyl-modified ASO binds to nuclear target mRNA in the liver and via RNase H2 initiates mRNA degradation	300 mg SC per week	1 Glomerulonephritis 2 Thrombocytopenia 3 Vitamin A deficiency	Vitamin A supplementation CBC, BMP & UA every 2 weeks	~\$450,000	Systolic strain imaging on echo compared to baseline at month 6
	Patisiran (<i>Onpattro</i>)	Phase 3 APOLLO-B NCT03997383	ATTRv & ATTRwt-CA NYHA I-III	siRNA which targets the 3' untranslated region of the TTR mRNA, forming the RISC and subsequent mRNA degradation	0.3 mg/kg IV infusion q 3 weeks (max dose 30mg)	1 Infusion reactions. 2 Vitamin A deficiency	Steroid IV, APAP, H1 & H2 blocker IV & Vitamin A Supplement	~\$450,000	Change from baseline at month 12 in 6-MWT
	Vutrisiran	Phase 3 HELLOS-B NCT04153149	ATTRv & ATTRwt-CA NYHA I-III	siRNA conjugated to GalNAc, binds to TTR mRNA in the nucleus and initiates mRNA degradation via RNase H2	25mg SC every 3 months	Unknown	Vitamin A supplement	Unknown	Composite outcome of all-cause mortality and recurrent CV hospitalizations at 30–36 months
	AKCEA-TTR-LRx / ION 682884	Phase 3 Cardio-TTRansform NCT04136171	ATTRv & ATTRwt-CA NYHA I-III	ASO conjugated to GalNAc, ASO portion shares the same base sequence as Inotersen, thus same mechanism of action	45mg SC every 4 weeks	Unknown	Vitamin A supplement Platelets every week BMP, LFTs and UPCR every 2 weeks	Unknown	Composite of CV mortality and frequency of CV clinical events at 120 weeks
	CRISPR (<i>NTLA-2001</i>)	Phase 1 Open label and Single Dose Expansion Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics	ATTRv-FAP	Cas9/CRISPR genome editing	IV dose escalation study	Unknown	None	Unknown	1 Adverse events 2 Change from baseline in serum TTR 3 Change from baseline in prealbumin 4 Pharmacokinetics 5 Pharmacodynamics

	Drug	Phase of study	Indication by Amyloid Type	Mechanism of action	Dose	Adverse Effects	Concomitant Therapy & Monitoring	Annual Cost	Primary Endpoints
									6 Change from baseline in anti-drug antibody and anti-Cas9 antibody levels
STABILIZERS	Tafamidis meglumine ⁶⁰ (<i>Vyndaqel</i>) Tafamidis free salt (<i>Vyndamax</i>)	Approved	ATTRv & ATTRwt-CA NYHA I-III	Benzoxazole derivative without NSAID activity which binds to T ₄ site on TTR	80mg po daily 61mg po daily	No safety signals of potential clinical concern	None	\$225,000	1 All-cause mortality lower with tafamidis (29.5% vs 42.9%) 2 Frequency of CV related hospitalizations lower with tafamidis (RRR 0.68)
	AG-10 (<i>Acoramidis</i>)	Phase 3 ATTRIBUTE-CM NCT03860935	ATTRv & ATTRwt-CA NYHA I-III	Mimics super-stabilizing activity of T119M, forms hydrogen bonds between neighboring serine residues at position 117 of each monomer	800 mg po twice daily	Unknown	None	Unknown	1 Change in 6MWT at 12 months 2 All-cause mortality and CV-related hospitalizations at 30 months
	Diflunisal ⁵⁷	Phase 2	ATTRv & ATTRwt-CA (off-label use)	NSAID, binds to T ₄ binding site on serum TTR	250mg BID po	1 Bleeding 2 Hypertension, 3 Fluid retention, 4 Renal dysfunction	Proton pump inhibitor Monitor CBC and BMP q 3-6 months	\$420	Safety and efficacy
	Tolcapone ⁶⁶	Phase 1 In-vitro and ex-vivo	ATTRv & ATTRwt-CA (V122I & V30M)	Cathecol-O-methyltransferase (COMT) inhibitor with a high affinity for T ₄ binding site on serum TTR	Unknown	Acute liver failure	LFTs at baseline and q 2-4 weeks for the first 6 months.	Unknown	No phase 3 trial to date
DEGRADATION / EXTRACTION	Doxycycline +/- TUDCA	Phase 2	AL-CA ^{#11} & ATTR-CA ¹¹²	Doxycycline – inhibition of MMP TUDCA – anti-amyloid fibril activity Synergistic activity to reduce amyloid fiber burden	Oral	Dermatologic Gastrointestinal	None	Unknown	AL-CA: 20% 1-yr mortality and 60% ASCT utilization with CvBoRD (no comparison arm) ¹¹¹ ATTR-CA: No significant changes in NYHA class, cardiac biomarkers or echocardiographic parameters over 22 months ¹¹²

	Drug	Phase of study	Indication by Amyloid Type	Mechanism of action	Dose	Adverse Effects	Concomitant Therapy & Monitoring	Annual Cost	Primary Endpoints
ANTI-SEEDING	Tab FH2	Pre-clinical	ATTRv	Peptide inhibitor which binds to the amyloid driving F- and H-stands of fragmented fibrils, thereby impeding self-recognition and seeding.	Pre-clinical	Unknown	Unknown	Unknown	Pre-clinical

ASO, antisense oligonucleotide; ATTRv, variant transthyretin amyloid; ATTRwt, wild-type ATTR; ATTR-FAP, ATTR familial amyloid polynuropathy; BMP, basic metabolic panel; CRISPR, clustered regularly interspaced short palindromic repeats; CV, cardiovascular; LFT, liver function test; MMP, matrix metalloproteinase; NSAID, non-steroidal anti-inflammatory; NYHA, New York Heart Association heart failure class; RISC, RNA-induced silencing complex; TUDCA, tauroursodeoxycholic acid; UPCR, urine protein-creatinine ratio.

* doxycycline alone

Table 4.

Therapies for Light Chain Cardiac Amyloidosis

Drug	Mechanism of action	Dose	Side Effects	Concomitant Therapy	Efficacy Data	
					Systemic Response	Organ response*
Melphalan ^{76, 122} (<i>Alkeran, Evomela</i>)	Alteration of guanine nucleotide, resulting in inter- and intra-strand DNA crosslinks, interfering with DNA replication and transcription	100 – 200 mg/m ² oral	Fluid retention Dizziness Gastrointestinal Myelosuppression Fever	Dexamethasone (ineligible for ASCT)	HR: • 51–76% [‡] CR: • 12–33% [‡]	18.7% cardiac response with dexamethasone (reduction in IVS of 2mm with resolution of HF) ¹²²
Cyclophosphamide (<i>Cytosan</i>)	Activity via the active metabolite, phosphoramide mustard, at the guanine N-7 position leading to cell apoptosis	100mg/d oral OR 300 mg/m ² oral weekly	Alopecia Hemorrhagic cystitis Gastrointestinal Myelosuppression Infection	Proteasome inhibitors CD38 monoclonal antibodies	See below under bortezomib	See below under bortezomib
Bendamustine ⁷⁹ (<i>Belrapzo, Bendeka, Treanda</i>)	Induces DNA interstrand crosslinks leading to cytotoxicity. Inhibits several mitotic checkpoints, promotes inefficient DNA repair	100 mg/m ² IV	Myelosuppression Fatigue Nausea / vomiting Fever	Dexamethasone	BDex: • CR 11% • VGPR 18% • PR 28%	BDex: • Overall organ response 29% • Cardiac response 13%
Lenalidomide ^{83, 84} (<i>Revlimid</i>)	<ul style="list-style-type: none"> • IL-6 inhibitors • Direct activation of caspase-8 mediated cell death • Active JNK to release pro-apoptotic proteins into the cytosol • Increase IL-2 expression and IFN-γ • Augment NK cell-dependent cytotoxicity 	~15 mg/d oral	Fluid retention Myelosuppression Elevated LFTs Infection Increases NT-proBNP Skin rash	Dexamethasone Melphalan Cyclophosphamide	HR : • 58–60% (RMD or RCD) CR: • 23% (RMD) • 11% (RCD)	Overall organ response: • 50% (RMD) • 29% (RCD) Cardiac response: • 40% (RMD) • 23% (RCD)
Pomalidomide ^{86, 123} (<i>Pomalys</i>)		2–4 mg/d oral	Fluid retention Infection Thromboembolism Skin rash Increases NT-proBNP	Dexamethasone	PomDex: • HR: 48–68% • VGPR: 18–25%	Overall organ Response 15% ¹²³ Cardiac response 15% ¹²³

Drug	Mechanism of action	Dose	Side Effects	Concomitant Therapy	Efficacy Data	
					Systemic Response	Organ response*
Daratumumab ^{100, 102} (<i>Darzalex</i>)	Human immunoglobulin G1 kappa (IgG1 κ) monoclonal antibody against CD38 antigen which leads to cell death via multiple mechanisms including complement mediated and antibody dependent cytotoxicity and apoptosis	1.8g SC	Infusion related reactions	Dexamethasone Bortezomib Cyclophosphamide (CyBorD)	HR • 76% • 92% (Dara-CyBorD) CR • 36% • 53% (Dara-CyBorD)	Cardiac response 42% with Dara-CyBorD
Corticosteroids	Bind to cytosolic glucocorticoid receptors and translocate to the nucleus where they modulate gene expression resulting in anti-inflammatory and immunosuppressive activity	20–40mg dexamethasone oral	Fluid retention Thrombosis Gastrointestinal hemorrhage Infection Psychosis	Used as part of a multidrug regimen	Poor hematologic response using high dose dexamethasone as a single agent	Unknown
Bortezomib ⁹⁰ (<i>Velcade</i>)	Proteasome inhibitor - reversible boronic acid inhibitor of the chymotrypsin-like activity to the proteasome	Variable dose and intervals, IV	Peripheral neuropathy	Dexamethasone Cyclophosphamide Melphalan	<u>CyBorD</u> ⁹⁰ : • HR 60% • CR 21%	<u>CyBorD</u> ⁹⁰ : • Overall cardiac response: 17% • By cardiac stage [‡] : – Stage II: 29% – Stage IIIa: 17% – Stage IIIb: 4%

* Cardiac response relies heavily on cardiac stage at diagnosis, thus direct comparison between studies is not possible.

[‡] Response varies with dexamethasone dose

[‡] Cardiac staging is based on Mayo 2004 criteria⁹¹

B, bortezomib; D, dexamethasone; CR, complete response; CYP, cyclophosphamide; HR, hematologic response rate; IFN-γ, interferon-gamma; JNK, c-jun terminal kinase; M, melphalan; NT-proBNP, N terminal pro brain natriuretic peptide; PR, partial response; VGPR, very good partial response;