AL amyloidosis: from molecular mechanisms to targeted therapies

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Systemic amyloidosis is caused by misfolding and extracellular deposition of circulating proteins as amyloid fibrils, resulting in the dysfunction of vital organs. The most common systemic amyloidosis, light-chain (AL) amyloidosis, is caused by misfolded light chains produced by a small, dangerous B-cell clone. The process of amyloid formation, organ targeting, and damage is multifaceted and, after disease initiation, the complexity of the downstream pathogenic cascade increases, rendering its control a challenge. Because of the progressive nature of the disease, early diagnosis to prevent end-stage organ damage is vital. Improving awareness and systematic use of biomarkers of organ damage in screening populations at risk may improve the still unsatisfactory diagnostic process. Amyloid imaging is now emerging as an important companion of biomarkers in formulating the diagnosis and prognosis and monitoring the effects of therapy. An accurate diagnosis is the basis for appropriate therapy that is risk-adapted and response-tailored. Effective treatments targeting the clone and rapidly and profoundly reducing the amyloid light chains have produced marked improvements in overall survival, making AL amyloidosis the most successful model of all amyloidoses. New therapies targeting the amyloid deposits are now under development, together with novel agents modulating light chain aggregation and proteotoxicity. The future of AL amyloidosis treatment is combination therapy and will require an innovative collaborative model for a rapid translation from bench to bedside with the ultimate aim of achieving a cure for this complex disease.

Learning Objectives

- Understand the complexity of the multifaceted process of amyloid formation and organ damage
- Understand that early and correct diagnosis is vital and that biomarkers and novel imaging techniques provide better tools for making the diagnosis and prognosis and for monitoring treatment efficacy
- Understand that the treatment goal is to improve cardiac function through the rapid and profound suppression of amyloidogenic light chain production. Future treatment will combine several approaches, including mainly precursor-depleting agents and antibodies promoting amyloid clearance

Introduction

For the Ham-Wasserman lecture, I have been given the privilege of providing my perspective on light-chain (AL) amyloidosis. I was initiated to the then-mysterious condition of amyloidosis by my 2 mentors, Jan Waldenström, who introduced me to the concept of "sick molecules and disease," and Elliott Osserman, with whom I had the opportunity to participate in the IV Symposium on Amyloidosis in 1984. At that conference, George Glenner reported the discovery of the protein forming cerebrovascular amyloid in Alzheimer disease. The cross-seeding of knowledge regarding cerebral and systemic amyloidosis has contributed to advancing the understanding of the molecular mechanisms of amyloid diseases, paving the way for new drugs. The most common form of systemic amyloidosis, AL amyloidosis, is caused by misfolded immunoglobulin light chains (LCs).

Here I share my experience of how AL amyloidosis has rapidly evolved from being a hopeless condition to a very treatable disease.

Amyloid and amyloidosis

Amyloid is constituted by highly ordered protein fibrils.[1] Several proteins can form amyloid fibrils, some of which are functional and some pathological.[2] A fascinating aspect of amyloid is that a wide variety of proteins can converge toward similar fibril structures that are associated with a broad range of functions, both physiological[3] and pathogenic (as in localized and systemic amyloid diseases). The unifying property of systemic amyloidoses is that the amyloidogenic protein is expressed in 1 or a few tissues, circulates in blood, and is finally deposited as amyloid fibrils in a number of target organs.[1] The process of amyloid development, starting from the misfolded protein to formation of prefibrillar aggregates and finally fibrils, results in cellular stress and death and subversion of the normal tissue architecture, leading to organ dysfunction and eventually death.[1,4] This is a complex process involving key players from the intracellular protein homeostasis (proteostasis)[5] network, extracellular chaperones and matrix components, metal ions, shear forces, limited proteolysis, and interactions with cells

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Off-label disclosure: Drugs approved for multiple myeloma are used off-label in AL amyloidosis.

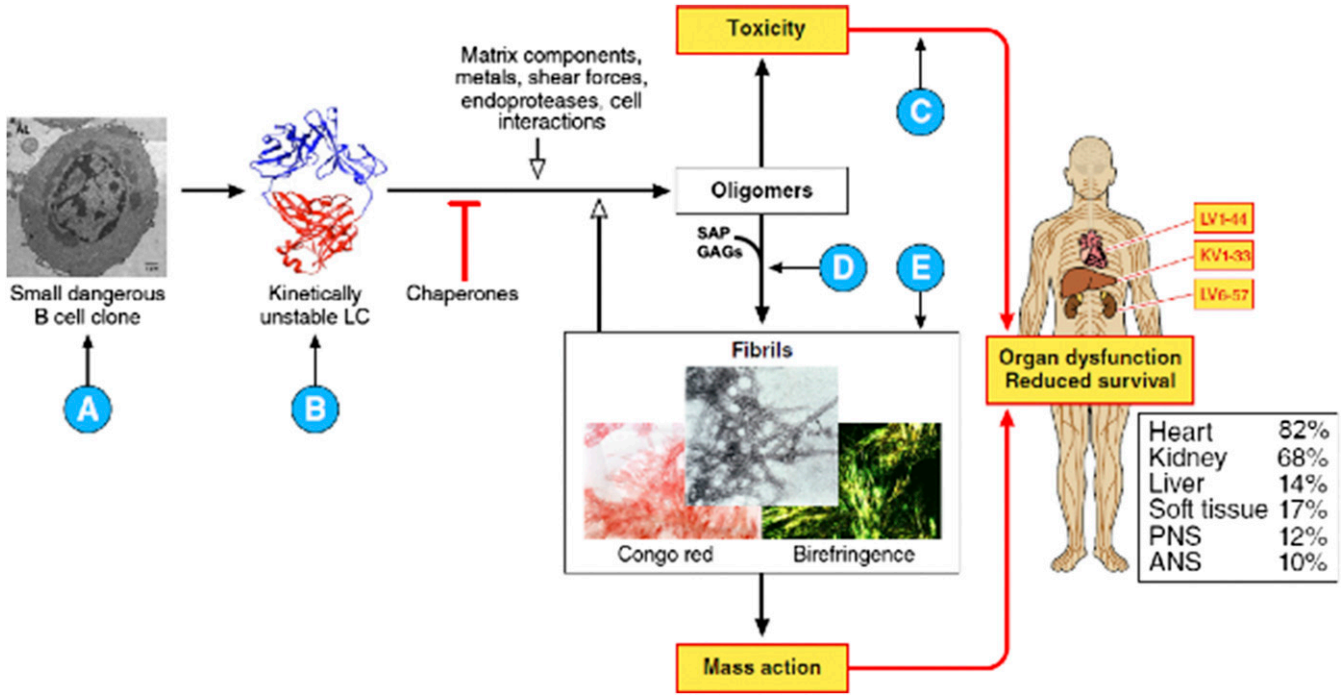

Figure 1. Molecular events leading to AL amyloidosis and possible targeted therapies. The usually small and indolent B-cell clone may produce a light chain (λ in ~80% of patients) with mutations in the variable region, causing low folding stability and high protein dynamics, favoring improper aggregation. Interactions with the microenvironment, such as extracellular chaperones, matrix components including glycosaminoglycans (GAGs) and collagen, shear forces, endoproteases, and metals modulate aggregation and oligomer formation. Cells may be transiently required in the amyloidogenic cascade and promote the initial nucleation of the deposits. The oligomers and, probably, the misfolded protein may exert toxic effects, impairing cell function and reducing cell viability in target organs. A propensity for certain germ line genes to target specific organs has been reported, for instance, LV1-44 for heart, LV6-57 for kidney, and KV1-33 for liver. Oligomers are on the pathway to forming highly organized fibrils displaying an X-ray cross-β fiber diffraction pattern. SAP is ubiquitously present in amyloid deposits. The formation of soluble amyloid oligomers can be catalyzed by amyloid fibrils. As seen by electron microscopy, the amyloid deposits are formed by rigid, nonbranching 10- to 12-nm width fibrils and exhibit a characteristic affinity for Congo red staining with birefringence under polarized light. Physical and mechanical replacement of parenchymal tissue by amyloid deposits cause dysfunction of vital organs. In addition, amyloid fibrils may contribute to cell toxicity. The frequencies of involvement of main target organs are derived from 1065 patients with AL amyloidosis diagnosed at our center between 2004 and 2015. The callouts indicate therapeutic targets. (A) The synthesis of amyloid light chain can be effectively suppressed by high-dose chemotherapy and conventional chemotherapy using drugs that target critical plasma cell functions and, more recently, by antiplasma cell immunotherapy. (B) Small molecules able to stabilize the light chain variable regions, thus inhibiting proteolysis, aggregation, and proteotoxicity, are under development. (C) Counteracting proteotoxicity caused by the misfolded light chains and aggregates is a challenging task that might be pursued with molecules modulating the mitochondria-mediated production of reactive oxygen species, as well as with metal-binding compounds. (D) Inhibitors of fibril formation have been tested in vitro, and compounds, such as epigallocatechin gallate, redirecting the oligomer off-pathway, are in clinical trials. (E) Immunotherapies, using different antibodies targeting diverse epitopes on amyloid fibrils, aiming at promoting the clearance of amyloid deposits are now being actively tested in the clinic. ANS, autonomous nervous system; PNS, peripheral nervous system.

(Figure 1). In certain systemic amyloidoses, cells are transiently required in the amyloidogenic cascade and promote the initial nucleation of the deposits. The amyloidogenic precursor may trigger amyloid formation when its concentration increases in body fluids and/or because a mutation favors misfolding. An imbalance between production and clearance of the amyloid precursor is probably the initiating factor in Alzheimer disease. A point mutation resulting in an amino acid substitution may be enough to destabilize the protein and make it prone to misfold and aggregate. Because nucleation and fibril formation are concentration-dependent, the pathogenesis of some of the systemic amyloidoses, such as reactive, AL, and wild-type β-2 microglobulin amyloidoses, includes an increased plasma concentration of the parent protein. Some wild-type proteins with an intrinsic amyloidogenic predisposition can, at a slow rate, form amyloid deposits that become symptomatic in the elderly (eg, wild-type transthyretin causing cardiac amyloidosis). It is not known why amyloid deposits accumulate and become symptomatic, usually in advanced age. Failures in the proteostatic machinery, both intracellularly and extracellularly; changes in the composition of the microenvironment and interactors; and a gradual weakening of repair mechanisms, associated with aging, have been advocated. Interactions with the extracellular environment may result in proteolytic cleavage and binding to matrix components such as glycosaminoglycans and collagen, which serve as scaffolds and facilitate aggregation and fibril buildup.[1,3] This was recently confirmed by our group through in situ characterization of protein aggregates in human tissues affected by AL amyloidosis by Fourier transform infrared microspectroscopy, which revealed a possible role of lipids (mainly cholesterol), collagen, and glycosaminoglycans in amyloid deposition in vivo.[6] Furthermore, several lines of evidence support a role for extracellular chaperones in the in vivo clearance of aggregation-prone extracellular proteins.[5] Finally, serum amyloid P (SAP) binds to amyloid fibrils and protects them from reabsorption.

As observed by electron microscopy, amyloid deposits are formed by 10- to 12-nm-wide nonbranching fibrils that display a cross-β fiber

diffraction pattern when examined with X-rays[2] and a characteristic affinity for Congo red staining with birefringence under polarized light.[1] The structural repetitiveness of the amyloid fiber provides an ideal template (seed) for replication and might therefore be transmissible between cells, or even infectious in the case of prion diseases. The kinetics of fibril formation has an *S*-shaped growth curve and a discernible lag phase. The lag phase can be drastically shortened and the process of amyloid formation accelerated by the presence of preformed fibrils, which can capture and catalyze the conversion of monomeric precursors, even at very low concentrations, into misfolded, toxic, and aggregation-competent structures. The kinetics of amyloid seeding has 3 important clinical implications: (1) early diagnosis is vital because the disease, once triggered, proceeds very rapidly; (2) it is essential to reduce the concentration of the amyloid precursor protein as deeply and as quickly as possible, because in the presence of amyloid fibrils, the process continues even at a very low concentration; and (3) clearance of the amyloid deposits synergizes with the reduction of the precursor to halt and reverse the accumulation of amyloid and accelerate recovery of organ function. Amyloid fibrils are generally resistant to degradation, although evidence suggests that amyloid does slowly resorb from the body once the amyloid precursor has been suppressed.

The amyloid protein: toward inhibiting aggregation and proteotoxicity

The biochemical and structural features of amyloidogenic LCs have been investigated to clarify the mechanism of amyloid formation. We, and others, found that destabilizing somatic mutations are present in both the complementarity-determining regions and framework regions and appear to increase the propensity of LCs to aggregate (reviewed in Blancas-Mejía and Ramirez-Alvarado[7]). There is evidence that amyloidogenic LC dimers are kinetically unstable (ie, unfold faster) and are thus susceptible to endoproteolysis, which results in the release of amyloidogenic LC fragments.[8] We recently undertook a systematic biophysical and structural characterization of a consistent set of patient-derived full-length LCs (n = 13) from patients with AL amyloidosis and multiple myeloma (MM). Based on a wide set of biophysical approaches, we found that folding stability and protein dynamics correlate with propensity to aggregation and proteotoxicity (unpublished). These findings suggest that molecules acting on the LC dimers, stabilizing the quaternary structure upon binding, could represent a good strategy for combating LC amyloidogenicity at its biophysical roots. Indeed, small-molecule ligands stabilizing LC variable domain (VL) dimers have recently been shown to inhibit amyloid formation.[9]

How does amyloid target and damage organ function?

The process of amyloid formation results in cellular injury, tissue damage, and organ dysfunction through mechanisms that are still incompletely understood. Organ dysfunction can result from the mass action exerted by amyloid deposits with disruption of tissue structure[10] and from proteotoxicity,[11] and the contribution of these 2 components varies among the different forms of amyloidosis.[1] Cellular toxicity can be determined by soluble amyloid oligomers, whose formation is catalyzed by amyloid fibrils. The latter can perturb cellular membranes and the process of fibril growth can contribute to toxicity. Furthermore, the cellular stress produced by the amyloid process may induce de novo aggregation and thus contributing to progression of the pathology.[2] Recently, it was reported that AL amyloid fibrils are cytotoxic at low concentrations, whereas soluble amyloid LCs induce apoptosis, suggesting that the mechanisms of cytotoxicity differ between soluble protein and amyloid aggregates.[12] The multitude of toxic species and damage mechanisms account for the multifaceted nature of amyloid diseases and suggest that targeting early steps of the amyloid process would provide the highest degree of therapeutic efficacy by eliminating toxic gains (or losses) of function and prevent the propagation of abnormal protein folding and aggregation.

Several researchers have hypothesized that organ tropism in AL amyloidosis may be a function of the LC variable region gene and gene family of the clone. Studies have shown important trends in LC variable region gene usage that confer a higher risk of involvement of specific organs. The germ line gene LV6-57 is more common in AL systemic amyloidosis than in the normal B-cell repertoire and is associated with renal involvement, whereas LV1-44 preferentially targets the heart and KV1-33 is associated with liver involvement.[13,14] These findings suggest that patients with LV6-57 monoclonal gammopathy, who can now be identified using mass spectrometry analysis of circulating free light chain (FLC), should be comprehensively evaluated for AL amyloidosis and followed closely using cardiac and renal biomarkers to timely detect the onset of the disease.[15]

Investigators have focused their efforts on unraveling the mechanisms of cardiac damage, because heart involvement is the determinant of survival in this disease. The development of experimental systems reproducing LC cardiotoxicity has been an important premise for better defining the molecular bases of tissue damage. Unfortunately, there is not a mouse model of AL cardiac amyloidosis, and the systems used in this setting include cultured human and rodent cardiac cells and the recently established animal models: *Caenorhabditis elegans,* developed by our group, and zebrafish, developed by Harvard investigators.[16,17] These systems share a crucial feature: that damage is exerted specifically by LCs that are cardiotropic in patients and not by nonamyloidogenic ones from MM. We have exploited the nematode *C. elegans* as a novel investigational tool because its pharynx, with autonomous contractile activity, is evolutionarily related to the vertebrate heart. The exposure to LCs from patients with cardiac amyloidosis produced persistent pharyngeal dysfunction and a significant reduction of the worms' lifespan.[16] The amyloid cardiotropic LCs caused severe functional and structural mitochondrial damage in the nematode, similar to that observed in amyloid-affected hearts from AL patients. We found that these effects were dependent on the presence of metal ions and that addition of metal-binding compounds blocked the production of reactive oxygen species and prevented the pharyngeal dysfunction caused by the amyloid LCs.[18] These compounds could be exploited in the clinic to inhibit LC proteotoxicity. Animal cardiac cells display a range of alterations that include increased production of reactive oxygen species, impaired intracellular calcium homeostasis, cellular contractile dysfunction, morphological damage of mitochondria, and reduced cell viability.[19-21] It is relevant that amyloid LCs purified from patients with amyloid cardiomyopathy induce p38 MAPK signaling[20]; this same pathway mediates type B natriuretic peptide (BNP) transcription, supporting a direct connection between LC cardiotoxic effects with induced MAPK signaling and BNP levels. This direct modulation of BNP synthesis by LC amyloid precursor means that the levels of BNP (and of its *N*-terminal prohormone, NT-proBNP) directly reflect the LC-induced cardiac pathology in AL amyloidosis. This pathogenic link is at the basis of the utility of NT-proBNP in the early diagnosis of cardiac involvement, in assessing the prognosis and in monitoring response to therapy in AL amyloidosis.[22]

Clinical evidence also supports an important role of amyloid LCs in determining cardiac damage. We showed that in patients with AL amyloidosis, variations in serum amyloidogenic free LCs translate into parallel changes in NT-proBNP.[23,24] Furthermore, the concentration of serum NT-proBNP is usually fivefold higher in AL amyloidosis than in transthyretin amyloidosis (ATTR) amyloidosis, and the survival of patients with AL amyloidosis and cardiac involvement is significantly poorer than that of patients with ATTR cardiomyopathy, despite a remarkably lower cardiac amyloid load in AL patients.[25]

The amyloidogenic clone: harnessing proteotoxicity

AL amyloidosis is caused by a small, but dangerous B-cell clone[26] that produces misfolded LCs that target practically all organs, possibly except the brain (Figure 1). The low incidence of AL amyloidosis and its typically low tumor burden, often masked by a polyclonal plasma cell (PC) background, account for the limited information on tumor cell biology, particularly when compared with MM. The amyloidogenic PC cell clone is characteristically small and indolent. When the clone accounts for more than 10% of the bone marrow cells, the prognosis is poor, similar to that of patients with AL amyloidosis associated with MM.[27] Clonal PCs in AL amyloidosis have similar phenotypic and copy number alterations profiles as those in MM, but their transcriptome is similar to that of normal PCs. Using a next-generation sequencing approach to investigate the mutational landscape of a cohort of patients with PC dyscrasias, it was found that the mutational pattern of AL amyloidosis is intermediate between those of monoclonal gammopathy of undetermined significance and MM.[28] Analysis of AL PCs with interphase fluorescence in situ hybridization (iFISH) showed that t(11;14) is the most commonly observed abnormality, being present in ~40% to 60% of patients.[29] It was reported that the survival of patients treated upfront with a bortezomib-containing regimen was inferior among those with the t(11;14) compared with that of patients who lacked this abnormality, with a hazard ratio of 3.1.[29] This finding was recently confirmed by a study in a large population of patients in which it was found that t(11;14)-positive patients also had a poor response and outcome after treatment based on immunomodulatory drugs.[30] Both high- and standard-dose melphalan regimens overcome the adverse impact of t(11;14), whereas trisomies are a poor prognostic feature in AL amyloidosis among melphalan- and bortezomib-treated patients. Furthermore, gain of 1q21 (present in almost one-quarter of patients) is associated with a poor response to melphalan-dexamethasone (MDex), which can be overcome by high-dose melphalan and bortezomib. Collectively, these findings highlight the importance of cytogenetic abnormalities on treatment outcome, and underscore the need to perform iFISH analysis to optimize treatment.

Clinical evidence suggests that AL PCs have an exquisite, intrinsic sensitivity to the first-in-class proteasome inhibitor, bortezomib. A recent study revealed distinctive organellar features and expression patterns, indicative of cellular oxidative stress, in primary AL PCs that showed an unprecedented intrinsic sensitivity to bortezomib, even higher than that of MM PCs. To test whether the sensitivity to proteasome inhibitor stems from production of misfolded LC, myeloma cell lines were engineered to express amyloidogenic and nonamyloidogenic LCs. We found that AL LC expression alters cell growth and proteostasis through proteotoxicity, and confers sensitivity to bortezomib.[31] Proteasome inhibitors are therefore targeted therapy in AL amyloidosis, and the discovery of this Achilles' heel of the amyloidogenic clone may direct future anticlone drug development.

Imaging amyloidosis

Amyloid deposits can be imaged exploiting the ubiquitous presence of SAP.[32] Imaging with radiolabeled SAP has enabled investigation of the kinetics of amyloid deposition and documented amyloid regression following effective suppression of the amyloid precursor, or, more recently, following anti-SAP immunotherapy. However, SAP imaging cannot detect amyloid cardiac involvement, and the quest to image the heart has been intense. Echocardiography is the mainstay for detecting and evaluating amyloid cardiac involvement. We developed a serine protease inhibitor (aprotinin) labeled with technetium 99 (^{99m}Tc) that effectively imaged the amyloid in the heart and could reveal "silent" early amyloid deposits in patients who later develop clinical symptoms.[33] Tracers used for imaging β amyloid protein in the brain (^{11}C-labeled Pittsburgh compound B [^{11}C-PIB],^{18}F-florbetapir, ^{18}F-florbetaben), with a very high sensitivity for amyloid, have been successfully applied to image cardiac AL amyloidosis. The bone-seeking radionuclide tracers ^{99m}Tc-3,3-diphosphono-1,2 propanodicarboxylic acid, ^{99m}Tc-hydroxymethylene diphosphonate, and ^{99m}Tc-pyrophosphate also seem to localize with remarkable sensitivity in cardiac ATTR deposits and can be used for differentiating AL amyloidosis from ATTR amyloidosis, particularly the wild-type form.[34] Cardiac magnetic resonance imaging has been an important development because it is easily available and has good specificity for the diagnosis of cardiac amyloidosis and can give accurate anatomical information, including the wall thickness and left ventricular mass. Equilibrium contrast magnetic resonance imaging allows quantification of the myocardial interstitial volume fraction, which is greatly expanded in amyloidosis, and can therefore be monitored.[35]

Clinical presentation

The overall sex- and age-adjusted incidence rate of AL amyloidosis per million person-years in a population-based study, published in 1992, was 10.5.[36]

The LC is λ in ~80% of patients. The disease usually involves all vital organs, possibly except the brain, so there can be damage to the heart (restrictive cardiomyopathy with heart failure and preserved ejection fraction, 82%), kidney (albuminuria evolving into renal failure, 68%), soft tissues (carpal tunnel syndrome, macroglossia, shoulder pad, and soft-tissue swelling/masses, 17%), liver (hepatomegaly, elevated concentrations of alkaline phosphatase and other liver function test abnormalities, 14%), peripheral and/or autonomic nervous system (sensory-motor axonal peripheral neuropathy, 12%, and autonomic dysfunctions such as orthostatic hypotension, erectile dysfunction and alternating constipation/diarrhea, 10%), and gastrointestinal tract (altered motility, bleeding, malabsorption, 8%). Cardiac involvement is the leading cause of morbidity and mortality in AL amyloidosis.[37] Because the amyloid process is associated with cytotoxicity and cell loss,[38] early diagnosis, anticipating irreversible end-organ damage, is of vital importance.

In 5% to 7% of patients, AL amyloidosis is associated with an underlying immunoglobulin M (IgM)-secreting lymphoplasmacytic lymphoma possibly with *MYD88* mutation. Compared with patients who have non-IgM AL amyloidosis, these patients are older, have a higher prevalence of neuropathy and lymph node involvement, and a lower proportion have cardiac involvement.

Table 1. Common proteins causing systemic amyloidosis and organ involvement

Designation	Parent protein	Organs involved
AL	Immunoglobulin light chain	Heart, kidney, soft tissues, liver, peripheral and/or autonomic nervous system, gastrointestinal tract
ATTR	Transthyretin (mutant)	Peripheral and autonomic nervous system, heart, eye, kidney, leptomeninges
	Transthyretin (wild-type)	Heart, ligaments, tenosynovium
AA	Serum amyloid A	Kidney, liver, heart, thyroid, autonomic nervous system, gastrointestinal tract
AApoAI	Apolipoprotein A-I (mutant)	Liver, kidney, testis, heart, peripheral nervous system
ALECT2	Leukocyte chemotactic factor-2	Kidney, liver

The biomarker revolution in diagnosis, prognosis, and assessment of treatment response

The availability of measurable biomarkers directly related to the pathogenesis of the disease has revolutionized the diagnosis and treatment of AL amyloidosis. The introduction of the FLC assay, which can be used to quantify and monitor the amyloid precursor, had an important impact on the diagnosis, risk stratification, and treatment follow-up.[39] In 2003, we discovered that NT-proBNP was the most sensitive biomarker of cardiac amyloidosis and a powerful prognostic determinant.[23] The possibility of measuring a biomarker of cardiac dysfunction opened a window on the mechanisms of heart injury caused by the amyloid process. Furthermore, NT-proBNP measurement allows early diagnosis, and constitutes the basis, together with troponin, for assessing the risk[40] and for evaluating the cardiac response to therapy.[41]

Diagnosis

That AL amyloidosis can affect almost any organ system, resulting in diverse clinical features that mimic other more common conditions of the elderly, leads to difficulties and delays in diagnosis (Table 1). A recent survey indicated that the diagnosis is usually made late, more than 1 year after the onset of symptoms in 40% of patients. Late diagnosis accounts for the high proportion (~25%) of subjects who present with advanced, irreversible cardiac damage and die within 12 months of diagnosis. Improving awareness is therefore crucial. The development of biomarkers of presymptomatic organ damage, NT-proBNP (Figure 2), with 100% diagnostic sensitivity in cardiac AL amyloidosis,[23] and albuminuria for renal involvement,[42] prompted us to advocate biomarker-based screening in patients with monoclonal gammopathy of undetermined significance and abnormal FLC ratio who are at higher risk of developing AL amyloidosis.[15,43] The conditions leading to a suspicion of systemic amyloidosis and the procedures required for diagnosis are summarized in Figure 3. Once the suspicion is raised, the diagnostic pathway is straightforward, with abdominal fat aspirate,[44] followed, if negative, by biopsy of labial salivary glands or of the involved organ, and Congo red staining showing diagnostic birefringence. Scintigraphy with bone tracers is essential for excluding wild-type transthyretin amyloidosis (ATTRwt), particularly in, but not limited to, elderly males with isolated heart involvement.[34] Approximately one-fifth of patients with ATTRwt present with a monoclonal protein and misdiagnosis between ATTRwt amyloidosis with AL amyloidosis could lead to inappropriate treatment. Unequivocal amyloid typing using mass spectrometry, immunoelectron microscopy, and immunohistochemistry in specialized laboratories is the basis for appropriate treatment (reviewed in Palladini and Merlini[45]). Gene sequencing is needed to rule out or confirm possible hereditary amyloidoses. Because of the small size of the PC clone, the identification of the amyloidogenic LCs requires the combination of immunofixation of both serum and urine and measurement of FLCs.[46] Recently, sensitive mass spectrometry–based technologies have been developed for monoclonal FLC detection and quantification. Bone marrow biopsies should be obtained, and iFISH analysis of PCs might offer guidance to treatment.

Prognostic factors

The systemic amyloid deposition with multiorgan involvement renders patients fragile and sensitive to treatment toxicity. Furthermore, patients' survival is extremely heterogeneous depending on the organs involved: patients with kidney involvement only may survive many years, whereas those presenting with advanced cardiac disease may succumb in a few weeks. Survival depends on 2 main factors: the extent of cardiac damage and PC burden/biology. In addition to the PC percentage and iFISH abnormalities already described, a difference between involved and uninvolved FLC (dFLC) concentrations >180 mg/L portends a poor prognosis.[47]

Cardiac involvement is the determinant of survival. The combination of NT-proBNP and cardiac troponin levels enables patients to be separated into 3 distinct risk groups; this staging system is now standard in clinical care for individual patient management and stratification in clinical trials.[40] Among stage III subjects, very high concentrations of NT-proBNP (>8500 ng/L) or hypotension identify patients with very advanced cardiac damage and survival restricted to a few weeks from diagnosis (stage IIIb).[48] Serum NT-proBNP concentration is sensitive to renal function, so serum BNP should be used in subjects with a low estimated glomerular filtration rate (<30 mL/min per 1.73 m^2).[49] The combination of levels of cardiac biomarkers with information on the load of the amyloid precursor provides a powerful revised staging system.[47] High-sensitivity cardiac troponin T is a strong predictor of survival[50] and allows stratification of patients into 3 stages. Other prognostic factors have been proposed and are awaiting validation, such as soluble suppression of tumorigenicity 2, growth differentiation factor 15, serum lactate dehydrogenase, the 6-minute walk test, New York Heart Association class, and baseline fatigue. Cardiac imaging also contributes to prognostication of survival. Echocardiographic left ventricular strain improves the predictive value of cardiac biomarkers,[51] and myocardial contraction fraction, which strongly correlates with left ventricular strain, has been proffered as a new independent prognostic factor. Using cardiac magnetic resonance, the extent of the late gadolinium enhancement provides incremental prognostic discrimination over cardiac biomarker stage in patients with AL cardiac amyloidosis.[35]

Although kidney involvement does not have a major impact on survival, it affects quality of life and access to effective treatments. We have developed and validated a staging system, based on estimated glomerular filtration rate and proteinuria, which is able to predict the risk of dialysis.[42] Recently, the ratio of 24-hour proteinuria to estimated glomerular filtration rate has been proposed as a marker of renal progression.

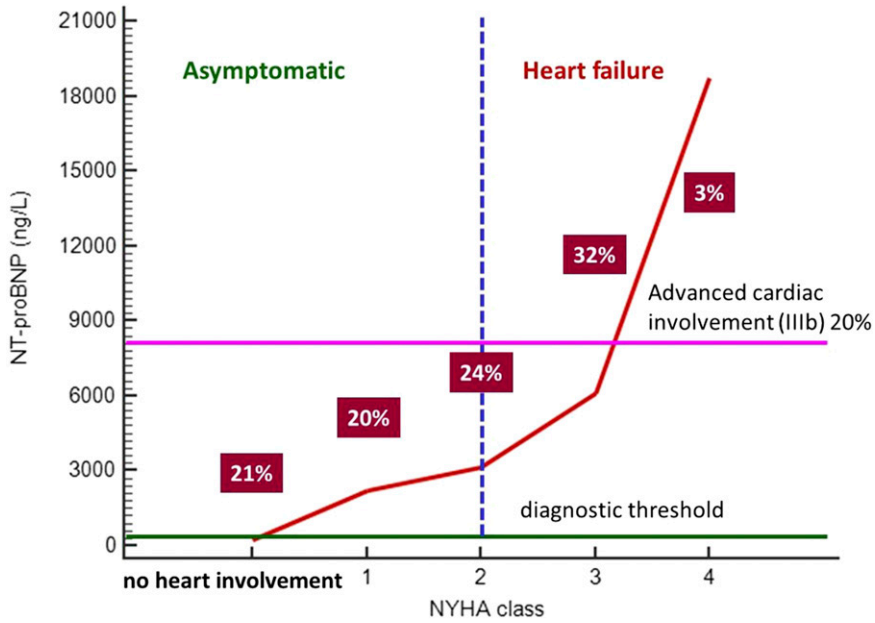

Figure 2. NT-proBNP increase above the threshold of 332 ng/L anticipates symptomatic cardiac failure. NT-proBNP concentration at diagnosis in 1065 patients is plotted versus cardiac symptoms (New York Heart Association [NYHA] class). The cardiac biomarker NT-proBNP can detect cardiac involvement before the appearance of symptoms of cardiac failure in one-fifth of patients. This biomarker could be used for screening patients for initial amyloid cardiac involvement in populations at risk to reduce the still-disheartening 20% of patients who are diagnosed with very advanced cardiac damage with NT-proBNP >8500 ng/L.

Monitoring treatment efficacy

Assessment of response comprises both evaluation of the efficacy of treatment in suppressing the supply of amyloid precursor protein and its effects on the function of the affected organs, which is essentially dependent on the former. The criteria for hematologic and organ (cardiac and renal) response have been validated through a huge international effort that we coordinated.[41,42] These criteria allow the timely identification of refractory patients and can be used as surrogate end points in clinical trials, allowing earlier study completion, thereby accelerating drug development.[22] The assessment of hematologic response is based on measurements of FLC. Patients who achieve a complete response (CR), defined as no detectable monoclonal protein band in serum or urine by immunofixation and normal serum FLC, or very good partial response (VGPR; ie, a reduction of dFLC to <40 mg/L) have the best outcomes.[41] The aim of treatment is, therefore, to achieve at least a VGPR. A robust measure of cardiac response is a decrease in serum concentration of NT-proBNP of 30% or 300 ng/L, which predicts survival and is a powerful guide to adequacy of treatment in AL amyloidosis. Frequent assessments of hematologic response are needed, with a view to immediately switching to potentially effective second-line treatment in patients who do not achieve a response. Recent data from our group indicate that further improvement of cardiac or renal function after CR can be achieved by obtaining a status of negativity for minimal residual disease, as assessed by next-generation flow cytometry.[52] In patients who do not have an improvement in organ function despite hematologic CR, studies for minimal residual disease are indicated and, if positive, further treatment aiming at minimal residual disease negativity may lead to organ response. According to current criteria, patients with a dFLC <50 mg/L cannot be assessed for response and are usually excluded from clinical trials. Patients with a low dFLC burden have distinct clinical features and significantly better survival. These patients may be evaluable for hematologic response with adapted criteria predicting improvement of overall and renal survival.[53,54]

Treatment

AL amyloidosis is the most successful example of effective therapy in the whole realm of amyloidosis. That amyloid LC is not produced constitutively, but by a small, indolent PC clone that can be controlled, or possibly, eradicated, is a boon. In AL amyloidosis, suppression of the synthesis of amyloid precursors, thus controlling the early events in the multifaceted cascade that leads to vital organ damage, dysfunction, and death, has translated into significant improvements of median survival that has nearly doubled over the past decade, with a significant proportion of patients (30% to 40%) now surviving more than 10 years.[55] Further improvements in the outcome are expected to be obtained from the combination of chemotherapy with immunotherapies promoting the reabsorption of amyloid deposits or targeting PCs. The aims of therapy are rapid elimination of the amyloid precursor and enhanced reabsorption of amyloid deposits, with the purpose of swiftly ameliorating cardiac function to improve patients' quality of life and extend their survival. The challenge is to provide the maximum tolerated effective therapy in a frail patient who needs a rapid and deep response. The reduced functional reserve of organs involved by amyloid magnifies the treatment toxicity in AL amyloidosis, making a risk-adapted approach to treatment critical.

Supportive care and organ transplantation

Supportive therapy is vital to gain time, palliating organ dysfunction, while chemotherapy exerts its efficacy. The multisystem involvement with cardiac, renal, and autonomic nervous system dysfunction requires close multidisciplinary monitoring. Sudden cardiac death is common in patients with cardiac AL amyloidosis and is usually caused by electromechanical dissociation. For this reason, implantable cardiac defibrillators, although they can successfully terminate arrhythmias,

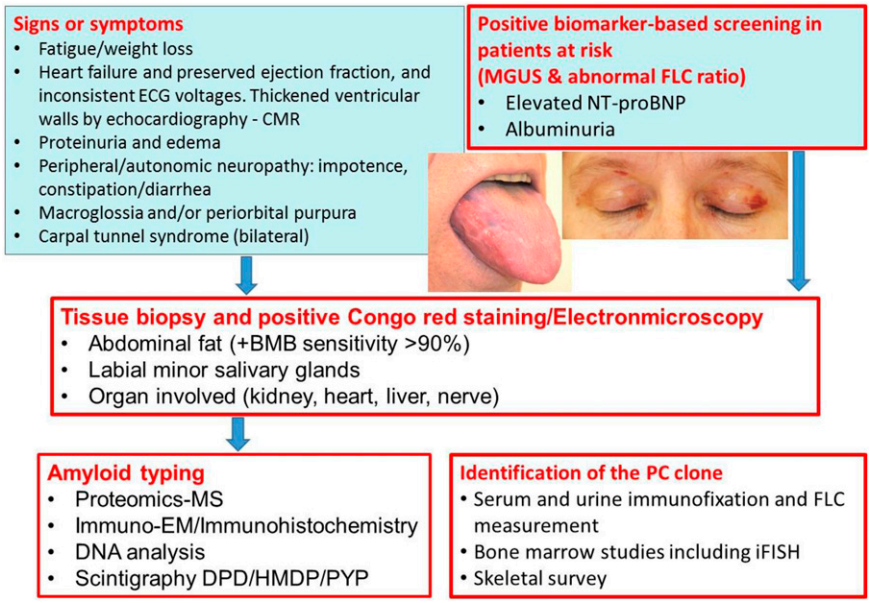

Figure 3. Diagnostic workup of systemic AL amyloidosis. AL amyloidosis can be suspected on the basis of symptoms of organ involvement or during follow-up of monoclonal gammopathy of undetermined significance using cardiac and renal biomarkers. Although some clinical signs, such as macroglossia and periorbital purpura, are very evocative of AL amyloidosis, they are rather uncommon, occurring in ~10% to 15% of patients, and they present very late in the disease course. Imaging is crucial for identifying heart involvement. The diagnosis is based on tissue biopsy. Less invasive biopsy sites (abdominal fat, minor salivary glands) should be preferred to organ biopsy. Amyloid deposits need to be unequivocally typed by reliable techniques. Imaging with bone-seeking tracers can detect ATTR cardiac amyloidosis and, in the absence of a monoclonal protein, may be diagnostic. The identification of the monoclonal light chains (~80% λ) requires sensitive techniques. BMB, bone marrow biopsy; CMR, cardiac magnetic resonance imaging; DPD, $_{99m}$Tc-3,3-diphosphono-1,2 propanodicarboxylic acid; EM, electron microscopy; HMDP, $_{99m}$Tc-hydroxymethylene diphosphonate; MS, mass spectrometry; PYP, ^{99m}Tc-pyrophosphate.

have not improved overall survival. Left ventricular assist devices are technically feasible for patients with severe heart failure from advanced cardiac amyloidosis, but their clinical utility is uncertain. Renal and cardiac transplants are indicated in patients who have persistent, advanced organ involvement despite hematologic CR. Cardiac transplantation can also be considered upfront in young patients with isolated severe heart involvement and should be followed by effective chemotherapy to prevent recurrence of amyloidosis in the graft.

Chemotherapy

Over the past decade, the remarkable progress in drugs for MM has been translated to AL amyloidosis, leading to improved treatment outcomes with extended survival.[55] Only a few controlled studies have been performed in AL amyloidosis, and a single, prospective randomized trial of novel agents has been recently completed.[56] Whenever possible, patients with AL amyloidosis should be treated within clinical trials. Table 2 reports the risk-adapted approach to front-line treatment.

Treatment of newly diagnosed patients

Early, controlled trials showed that chemotherapy using melphalan and prednisone can produce hematologic responses and prolong survival. However, the response rates were low, and overall survival remained dismal. The introduction of autologous stem cell transplantation (ASCT) marked a major step forward in the treatment of AL amyloidosis.[57] Patients with AL amyloidosis who achieve a CR have superior outcomes to those with myeloma, indicating that the biology of the AL PCs is different. Cardiac biomarkers play a central role in the assessment of eligibility for ASCT and contributed greatly to reducing treatment-related mortality to the present level of ~5%.[58] Compared with full-intensity melphalan conditioning, reduced-dose conditioning is associated with significantly reduced response rates and overall and progression-free survival.[59] The increase in the availability of effective therapies for AL amyloidosis may help in the decision to omit the use of attenuated conditioning for ASCT in this disease and rather to choose standard treatments.[59] Although a randomized trial failed to prove the superiority of ASCT over conventional MDex treatment,[60] data from several uncontrolled studies showed excellent clonal response rates to ASCT. The hematologic response rate to ASCT exceeds 70%, with ~35% of patients obtaining a CR and a median survival approaching 8 years, with about 55% of patients in CR projected to be alive at 14 years, raising the hope that a proportion of these patients might be cured.[58,61] In patients with >10% PCs in the bone marrow, induction therapy with bortezomib-based regimens significantly improve the quality of response after ASCT. Consolidation with bortezomib-based regimens in subjects who fail to achieve a CR after ASCT increases the CR rate to almost 60%. However, only 15% to 20% of patients with AL amyloidosis are at low risk and eligible for ASCT, whereas ~60% are at intermediate risk. At our center, the standard upfront treatment of intermediate-risk patients has been oral MDex, which we introduced in 2004.[62] We have updated our experience with this regimen. With a median follow-up of 6 years, overall median survival was 7.4 years. The hematologic response rate was 76%, with 31% of patients obtaining CR, and projected survival of patients in CR after MDex is more than 80% at 7 years.[63] However, data on very long-term outcome are still lacking.

The introduction of bortezomib in the treatment of AL amyloidosis was a major breakthrough with high response rates to the drug given as a single agent,[64] and unprecedented hematologic response rates (up to 94%, with 71% CRs) in patients receiving the combination of

Table 2. Outcome of AL amyloidosis treated with selected upfront regimens according to disease severity

Disease severity	Treatment	Patients	Hematologic response % (CR %, VGPR %)	Organ response %	PFS (median months)*	Overall survival (median years)
Fit patients (15%–20%), age <65 y, stage I/early II, NT-proBNP <5000 ng/L, cTnT <0.06 ng/mL, ECOG 0-1, eGFR >50 mL/min, no gastrointestinal bleeding	ASCT[58]	1536	After 2007 71 (37, —)	After 2007 K 32	NR	68% at 5 y
	ASCT[61]	629	— (35, —) 45 CR with Mel 200 34 CR with Mel 100-140	—	NR	7.6
Intermediate fit (50%–60%), stage II and stage IIIa, ECOG 1-2, SBP >100 mm Hg, NT-proBNP <8500 ng/L	CyBorD[68]	128 Stages II and IIIa	66 (20, 27)	H 22 K 25	13	5
	BMDex[66]	87 (19 stage IIIb)	69 (42, 13)	H 16 K 16	39	53% at 5 y
	MDex[63]	119 (12 stage IIIb)	76 (31, 29)	H 37 K 24	30	7.4
Frail (15%–20%), stage IIIb, NT-proBNP >8500 ng/L, SBP <100 mm, ECOG 4, NYHA 3–4	CyBorD attenuated[68]	43	42 (14, 9)	H 4	NR	7 mo
	MDex attenuated†	62	37 (9, 15)	H 18	NR	7 mo

cTnT, cardiac troponin T; ECOG, Eastern Cooperative Oncology Group performance status; eGFR, estimated glomerular filtration rate; H, heart; K, kidney; NR, not reported; NYHA, New York Heart Association classes of heart failure; PFS, progression-free survival; SBP, systolic blood pressure.
*There are no validated progression criteria in AL amyloidosis and PFS is defined differently in different studies.
†Data unpublished.

cyclophosphamide-bortezomib-dexamethasone (CyBorD).[65] Two retrospective, matched case-control studies confirmed higher response rates with bortezomib-containing regimens (BMDex and CyBorD) compared with standard MDex or cyclophosphamide/thalidomide/dexamethasone, although without an advantage in overall survival.[66,67] It is noteworthy that patients with high LC burden (>180 mg/L) respond significantly better to a BMDex regimen than to MDex or CyBorD. We recently reported the largest, real-world series of patients treated with CyBorD: the overall response rate was 60%, with 23% of the patients achieving a CR. Response rates decreased with increasing cardiac stage (Table 2) as a result of early deaths and because of the necessity to attenuate the dose.[68] We have coordinated an international, randomized phase 3 study comparing MDex and BMDex (www.clinicaltrials.gov #NCT01277016) that has been now completed: an interim analysis showed that BMDex induced a VGPR or better in 62% of patients, compared with 40% with MDex ($P = .016$), with ~40% cardiac and renal responses.[56]

The combination of lenalidomide, melphalan, and dexamethasone was reported to be effective at achieving hematologic remission, organ response, and prolonged survival in transplant-ineligible, newly diagnosed patients. Hematologic and cardiac toxicities were the predominant adverse events, and strict surveillance is required.[69,70]

Although no controlled trials are yet available to make an evidence-based decision, indications derived from the treatment outcome based on genetic abnormalities detected by iFISH, described previously, and clinical considerations help in optimizing the choice of therapy in fit and intermediate fit patients. Considering the lower response rate to bortezomib-based regimens in the 40% to 60% of patients with t(11;14), regimens containing melphalan at conventional dosage (MDex or BMDex) or high dosage (ASCT) should be favored in such subjects. Patients with 1q deletion are best treated with bortezomib-based regimens. Patients who are considered potential candidates for ASCT should be treated with CyBorD to preserve stem cells. Patients with severe neuropathy should avoid bortezomib and are probably best treated with MDex. Finally, patients who present with high dFLC (>180 mg/L) could benefit from BMDex.

Improving the outcome of the ~20% frail patients who present with very advanced cardiac involvement (stage IIIb) is an urgent priority (Table 2). These are the patients most in need of rapidly effective therapy, and yet they are the most sensitive to treatment side effects. Cautious weekly treatment escalation of dexamethasone and bortezomib under close monitoring is recommended. Approximately 40% of these patients obtain a response to reduced-intensity combination therapy, achieving a prolonged survival.[68] Cardiac transplantation should be considered in eligible patients in stage IIIb, whereas the role of left ventricular assist devices needs further investigations.

Treatment of relapsed/refractory patients

To determine the best time to start therapy in patients who relapse, we analyzed 259 patients who responded to upfront therapy (unpublished data). Ninety-two patients (35%) needed second-line therapy after a median of 49 months. Cardiac and renal progression were observed in 22% and 12% of patients, respectively, who received second-line therapy. Patients who had cardiac progression (determined by NT-proBNP levels) had a median survival of 17 months compared with 62 months ($P = .002$) in those whose cardiac involvement did not progress, indicating that second-line treatment should start promptly at progression of FLC, before cardiac progression has occurred. A recent analysis of the treatment patterns and outcome following initial relapse or refractory disease in patients with AL amyloidosis showed that the time to next therapy was significantly reduced in patients retreated with the same therapy compared with that in patients treated with a different therapy (22 months vs 32.3 months, respectively; $P = .01$), but there was no impact on survival (30.8 months vs 51.1 months; $P = .5$). The type of therapy at relapse (ASCT vs proteasome inhibitor vs immunomodulatory drug vs melphalan vs steroids and others) did not alter

Table 3. Outcome of relapsed/refractory AL amyloidosis patients who received salvage therapy with different treatment regimens

Treatment	No. of patients	Hematologic response % (CR %/VGPR %)	Organ response %	Median PFS	Median overall survival
Bortezomib[64]	70	60 (24/—)	H 13, K 29	77% at 1 y	90% at 1 y
Lenalidomide-dexamethasone[90]	84	61 (20/8)	H 12; K 40	73% at 2 y	84% at 2 y
Pomalidomide-dexamethasone[74]	28	68 (4/25)	K 17	16 mo	26 mo
Bendamustine[77]	125*	36 (2/8)	H 13; K 15	NR	21 mo
Ixazomib[75]	27	52 (10/33)	H 45; K 45	15 mo	85% at 1 y
Carfilzomib[76]	24	63 (13/33)	5 (21%): 3 K, 1 GI, 1 liver	20 mo	NR
Daratumumab[80]	25	76 (36/24)	NR	NR	NR

GI, gastrointestinal tract.
*Twelve patients were upfront cases.

the time to next therapy, or survival from the start of the second-line treatment.[71]

Immunomodulatory drugs have been used successfully in refractory/relapsing patients (Table 3). Lenalidomide and pomalidomide proved able to overcome resistance to alkylating agents, bortezomib, and thalidomide, with overall hematologic response rates ranging from 40% to 60%, but with low rates of CR.[72-74] Lenalidomide should be used with caution in patients with substantial proteinuria because it is associated with renal failure. An increase in NT-proBNP has been reported in patients taking immunomodulatory drugs, a fact that should be considered when assessing cardiac response. Pomalidomide/dexamethasone can rescue patients with AL amyloidosis previously exposed to alkylators, proteasome inhibitors, and lenalidomide.[73] We recently reported that responses to pomalidomide/dexamethasone are frequent, rapid, and improve survival, suggesting the use of this agent in front-line, combined therapy.[74]

Novel drugs targeting amyloid PCs

The care of patients with AL amyloidosis is benefiting from the recent explosion of novel drugs in MM.

The second-generation oral proteasome inhibitor ixazomib has been tested in a phase 1/2 trial in 27 relapsed/refractory patients with AL amyloidosis. Overall, the hematologic response rate was 52% and organ responses were seen in 56% patients (5 cardiac, 5 renal). The median hematologic progression-free survival was 15 months; 1-year progression-free and overall survival rates were 60% and 85%, respectively (Table 3).[75] These encouraging results prompted a randomized phase 3 trial, which is currently under way, comparing ixazomib with physician's best choice of management (#NCT01659658). A phase 1/2 study assessing safety and efficacy of a second-generation proteasome inhibitor, carfilzomib, in 24 evaluable, previously treated patients with AL amyloidosis, showed a good efficacy, with a 63% response rate, including 3 CRs, but also important grade 3/4 cardiopulmonary toxicities in 36% of patients.[76] An additional option for relapsed/refractory patients is bendamustine. A recent collaborative study, including 125 mostly pretreated patients, of bendamustine and oral prednisone (plus rituximab in 35 patients with AL associated with IgM monoclonal protein), showed a hematologic response rate of 36% (CR 2%, VGPR 8%).[77]

Immunotherapy is now an important component of MM treatment.[78] In AL amyloidosis, the anti-CD38 antibody, daratumumab, showed efficacy in 2 heavily pretreated patients.[79] A recent study including 25 heavily pretreated patients showed a response rate of 76%, including CR in 36% and VGPR in 24%.[80] Most likely daratumumab will soon move to front-line therapy, possibly in combination with immunomodulatory drugs, with pomalidomide being a good candidate considering its rapidity of action, or with oral proteasome inhibitors. Other immunotherapy approaches, such as elotuzumab, a humanized antibody that targets signaling lymphocytic activation molecule family member 7 (SLAMF7), has proven effective in association with lenalidomide and dexamethasone in MM and awaits testing in AL amyloidosis. Immune checkpoint inhibitors and chimeric antigen receptor T-cell therapy are being developed in MM and will probably be explored in AL amyloidosis in the near future.

Targeting the synthesis of amyloid LC, its toxicity, and amyloid formation

RNA interference targeting the LC constant region has been explored in preclinical models.[81] The possibility of exploiting intracellular quality control mechanisms to selectively reduce the secretion of misfolded LCs has been recently investigated in vitro. Small ligands can kinetically stabilize the amyloid LC, similarly to the kinetic stabilizers developed in ATTR amyloidosis, with inhibition of aggregation and amyloid formation.[9]

We reported that a small molecule, the anthracycline 4′-iodo-4′-deoxy-doxorubicin, inhibited amyloidogenesis in vitro and facilitated the clearance of amyloid deposits in subjects with AL amyloidosis, improving the clinical status.[82,83] In a small, multicenter, phase 2 trial of 4′-iodo-4′-deoxy-doxorubicin, using a probably inappropriately low dosage of the drug (15 mg/m^2), the organ response rate was 15%.[84] Doxycycline, an antibiotic with a molecular structure closely resembling that of 4′-iodo-4′-deoxy-doxorubicin, is also able to interfere with amyloid fibril formation in a mouse model of AL amyloidosis, and abrogates LC toxicity in the *C. elegans* model.[16] This is relevant to the recent report on retrospectively determined outcomes of adding doxycycline to standard chemotherapy in 30 patients with cardiac AL amyloidosis compared with 73 cardiac stage-matched controls. The addition of doxycycline to chemotherapy reduced the mortality in cardiac AL amyloidosis, possibly reducing LC proteotoxicity. The benefit of doxycycline appeared greatest in patients with Mayo stage IIIa disease and was limited, if any, in the very advanced stage IIIb patients.[85]

Interest in polyphenols (epigallocatechin gallate) as inhibitors of fibrillogenesis was triggered by the case of a patient whose cardiac symptoms improved after purposely drinking large amounts of green tea. In a retrospective case series, including 59 patients with cardiac involvement who regularly consumed green tea, 11 patients had at

least a 2-mm decrease of interventricular wall thickness.[86] Controlled clinical trials are under way also at our center to test the activity of epigallocatechin gallate (#NCT01511263, #NCT02015312).

Targeting the amyloid deposits with immunotherapy

Passive immunotherapy in AL amyloidosis has been explored using diverse antibodies. The infusion of an anti-LC monoclonal antibody 11-1F4 with specificity for an amyloid-related epitope caused the reduction of subcutaneous amyloidomas in mice produced by injecting amyloid extracts from patients' livers and spleens. A pilot study performed on 21 patients, previously successfully treated with chemotherapy, reported organ responses in 12 of the 18 evaluable patients.[87] The obligate combination of a palindromic compound, CPHPC, which efficiently depletes SAP from the plasma but leaves some SAP in amyloid deposits, with a humanized monoclonal IgG1 anti-SAP antibody resulted in a marked reduction of visceral amyloid deposits in a phase 1 clinical trial.[88] Repeated administration of the anti-SAP antibody progressively removed amyloid from liver and other organs including the kidney. A phase 2 trial in patients with amyloid cardiomyopathy is ongoing (#NCT03044353). A monoclonal antibody (NEOD001) targeting an LC cryptic epitope exposed on amyloid fibrils has been reported to accelerate the regression of AL κ amyloidomas in mice. The phase 1/2 study of NEOD001, involving 27 patients with AL amyloidosis who achieved a partial hematologic response or better to previous chemotherapy, found cardiac and renal response rates of 57% and 60%, respectively.[89] A recent update on the expansion cohort of a phase 1/2 study, including 69 patients, showed that 53% of patients obtained a cardiac response, 64% a renal response and, notably, 82% had a neurological response (peripheral neuropathy). A phase 3 (#NCT02312206) and a phase 2b study (#NCT02632786) with this antibody are nearly completed. Interestingly, the studies targeting the amyloid deposits have been performed in patients in whom the control of the production of the amyloid LC was previously achieved through chemotherapy, indicating that the future of therapy in AL amyloidosis will be based on combined approaches.

Conclusions

It is a bright era for AL amyloidosis, with several novel therapies in the pipeline. We need to accelerate the pace of drug development and create new models involving coordinated collaboration among academia, pharma, private foundations, government funding and regulatory agencies, patients, and caregivers as key partners, knowledge resources, and decision-makers. Biomarkers and imaging can be integrated into innovative trial designs to accelerate the clinical development of novel medicines. Early diagnosis remains vital for improving treatment efficacy and overall survival. Earlier diagnosis and better treatment options have produced a reduction in early deaths and improved the tail end of the survival curves in the past few years. With greater awareness of the disease and additional new drugs to target bone marrow PCs and the amyloid deposits, further advances are expected in the near future.

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