Systemic immunoglobulin light chain amyloidosis

Giampaolo Merlini[1,2], Angela Dispenzieri[3], Vaishali Sanchorawala[4], Stefan O. Schönland[5], Giovanni Palladini[1,2], Philip N. Hawkins[6], and Morie A. Gertz[3].

1. Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
2. Department of Molecular Medicine, University of Pavia, Pavia, Italy
3. Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN, USA
4. Section of Hematology-Oncology and Amyloidosis Center, Boston University School of Medicine, Boston, MA, USA
5. Department of Internal Medicine V (Haematology, Oncology and Rheumatology), Amyloidosis Center, University Hospital Heidelberg, Heidelberg, Germany
6. National Amyloidosis Centre, University College London (Royal Free Campus), London, UK

Correspondence:
Giampaolo Merlini e-mail: gmerlini@unipv.it

Abstract

Systemic immunoglobulin light chain (AL) amyloidosis is a protein misfolding disease that is caused by the conversion of immunoglobulin light chains from their soluble functional states into highly organized amyloid fibrillar aggregates that lead to organ dysfunction. The disease is progressive and, accordingly, early diagnosis is vital to prevent irreversible organ damage, of which cardiac and renal damage predominate. The development of novel sensitive biomarkers and imaging technologies for the detection and quantification of organ involvement and damage are facilitating earlier diagnosis and enhanced evaluation of the efficacy of new and existing therapies. Treatment is guided by risk assessment which is based on levels of cardiac biomarkers; close monitoring of clonal and organ response guides duration of therapy and changes in regimen. Several new classes of drugs, such as proteasome inhibitors and immunomodulatory drugs, along with high-dose chemotherapy and autologous haematopoietic stem cell transplantation, have led to rapid and deep suppression of the amyloid light chain production in the majority of patients. However, effective therapies for patients with advanced cardiac involvement are an unmet need. Passive immunotherapies targeting clonal plasma cells and directly accelerating removal of amyloid deposits promise to further improve the overall outlook of this increasingly treatable disease.

[H1] Introduction

Amyloidosis is a group of complex diseases that are caused by protein misfolding and aggregation into highly ordered amyloid fibrils that deposit in tissues, resulting in progressive organ damage. These amyloid fibrils are characterized by a cross-β-sheet quaternary structure[1]. Over time, protein misfolding and amyloid accumulation can result in severe organ dysfunction. Protein aggregates, or preceding intermediaries, may induce cell dysfunction and death, a process termed proteotoxicity. In addition, the distortion of tissue architecture caused by amyloid deposits contributes to organ dysfunction [2]; however, these mechanisms are poorly characterized.

To date, 36 proteins that can form extracellular amyloid fibrils in humans have been identified: some form localized deposits, such as β-amyloid in Alzheimer disease, leading to localized amyloidosis, and others accumulate throughout the tissues of the body (known as systemic amyloidosis)[3]. At least 17 proteins can cause systemic amyloidosis;

immunoglobulin heavy or light chains are notable in being able to form both systemic amyloid deposits or localized amyloid deposits, for example those restricted particularly to urothelial tissue and the larynx[4]. Systemic amyloidosis can be hereditary or acquired; the two most common forms of systemic amyloidosis — monoclonal immunoglobulin light chain (AL) amyloidosis and wild-type transthyretin (ATTR) amyloidosis— are acquired. AL amyloidosis is typically found in individuals with monoclonal gammopathy, and is caused by the increased production of immunoglobulin light chains owing to the proliferation of clonal plasma cells that characterize these disorders; these light chains aggregate into amyloid fibrils, leading to organ damage. Wild-type ATTR amyloidosis is caused by the aggregation of transthyretin, is age related and predominantly affects men >70 years of age. Another form of non-hereditary systemic amyloidosis is caused by persistent high concentrations of serum amyloid A protein (an acute phase reactant) associated with chronic inflammation caused by chronic inflammatory disorders such as rheumatoid arthritis, persistent infections, or hereditary autoinflammatory diseases (familial Mediterranean fever, cryopyrin associated periodic syndrome and many others)[5]. Systemic amyloidosis caused by leukocyte chemotactic factor 2, mainly presents with nephropathy and is gaining greater recognition in the United States[6].

Systemic amyloidosis can also be caused by genetic mutations inherited in an autosomal dominant manner. More than 120 point mutations in *TTR* (encoding transthyretin) can cause systemic amyloidosis that mainly affects the peripheral nervous system and the heart. Genetic variants of *APOA1, APOA2, APOC2* and *APOC3* (encoding apolipoprotein AI, AII, CII and CIII, respectively), as well as *FGA* (encoding fibrinogen alpha chain), *GSN* (encoding gelsolin), *CST3* (encoding cystatin C) and *LYZ* (encoding lysozyme) can also cause hereditary systemic amyloidosis [3] (Table 1).

Despite the biochemical and aetiological heterogeneity of systemic amyloidosis, the clinical manifestations of the different forms largely overlap and essentially depend upon the affected organs. Predominantly affected organs include the kidney and heart, followed by the peripheral nervous system (including the autonomic nervous system), liver, gastrointestinal tract and soft tissues. Cardiac damage is a major determinant of survival and, therefore, a major goal of therapy is to improve cardiac function. A rapid and profound decrease of the amyloid precursor protein can reverse organ dysfunction, and is the aim of therapy. In AL amyloidosis, therapy should target the B cell clone responsible for producing the aberrant clonal immunoglobulin protein. The type and intensity of

treatment targeting the B-cell disease is based on risk assessment, which is based on the characteristics of the patient and the biology of the clone. Immunotherapies targeting the amyloid clone or the amyloid deposits are now in development; hopefully, these new agents will enter clinical practice, and will be combined with therapies to suppress the amyloid protein, which might improve quality of life (QOL) and improve survival.

This primer focuses on systemic AL amyloidosis, highlighting the disease mechanisms and basis for effective treatment, owing to advance in deciphering the molecular mechanisms of this form of amyloidosis and in developing novel, effective therapies that have improved quality of life and survival[7, 8].

[H1] Epidemiology

Limited data on the epidemiology of AL amyloidosis are available due to the lack of large population databases to assess incidence. The prevalence of the disease rises with increasing age. The prevalence doubles from age 35-54 compared to 65+ with reported mean age of 63, and 55% of patients are men [9]. There are two known risk factors for AL amyloidosis. The first is a pre-existing monoclonal gammopathy. Among patients with MGUS, the relative risk is 8.8[10]. In one series of 1384 MGUS patients followed for up to 50 years, 14 developed AL amyloidosis (1%). As many as 10-15% of patients with myeloma have overt, coexisting AL amyloidosis, and in another series as many as 38% of myeloma patients were found to have covert co-existing AL amyloidosis[11]. Approximately 1% of patients with pre-existing myeloma, who are not diagnosed with AL amyloidosis simultaneously, will go onto to develop AL amyloidosis[12]. Antecedent viral infection does not appear to be a predisposing factor. The other identified risk factor is the existence of particular single nucleotide polymorphisms (SNPs). Associations were found in a genome-wide association study (GWAS) on 1229 AL amyloidosis patients. SNPs at 10 loci showed evidence of an association at $P<10^{-5}$. Rs9344 at the splice site of *cyclin D1*, which promotes translocation (11;14), reached the highest significance, $P=7.80 \times 10^{-11}$. The SNP rs79419269, which is close to gene *SMARCD3* which is involved in chromatin remodeling was also significant ($P=5.2 \times 10^{-8}$). These data provide evidence for common genetic susceptibility to AL amyloidosis[13].

[H2] *Incidence of AL amyloidosis*

Six studies have evaluated the incidence of systemic AL amyloidosis, all of which were

carried out in the Americas and Europe[14,15, 16,17-20] (Table 2). The first study was carried out using the Olmsted County Project in Minnesota, USA, and reported an overall sex-adjusted and age-adjusted rate of 8.9 cases per million person-years between 1950 and 1989 and 10.5 cases of systemic AL amyloidosis per million person-years between 1970 and 1989[14]. An update to this study that included patients from the same region between 1990 and 2015, demonstrated an incidence of 12 cases per million per year, which did not significantly differ from that reported in the earlier study[20] (Kyle et al Accepted Mayo Clinic Proc.). The only other true population-based study of the incidence of systemic AL amyloidosis was carried out in the Limousin region of France from 2012 to 2016 [18]. This study demonstrated a crude yearly incidence of 12.5 cases per million inhabitants over the 5 year period studied. The four other studies were not true population-based studies, and ascertained the incidence based on death certificate reports and hospital discharges, among other methods[15,17,16, 19]. One study extrapolated the incidence from referral rates to the UK National Amyloidosis Centre, death certificates, and the distribution of types of systemic amyloidosis cases seen at the center[15] and estimated an incidence of at least 3 cases per million person-years in England in 2008. A study in Sweden used myeloma statistics and amyloid hospital discharge diagnoses to derive an annual incidence of 3 cases per million person-years between 2001 and 2018[16]. An incidence of 6.1 per million person-years adjusted for the population of Buenos Aires (2010 census) based on 12 persons with AL amyloidosis [19]. These investigators designed a prospective cohort of all members of a prepaid HMO in Buenos Aires between 2006 and 2015. They calculated the number of incident cases of amyloidosis per one million person-years and adjusted it using the Buenos Aires Census of 2010. Lastly, another study estimated the incidence of AL amyloidosis using US claims data between 2007 and 2015[17], and reported an age-adjusted and gender-adjusted incidence of 10.8-15.2 cases per million person-years. However, this estimate might be high as this study differentiated patients with AL amyloidosis from other forms of amyloidosis based on the receipt of 'AL amyloidosis defining therapies', which included therapies that are not specific for AL amyloidosis. Doxycycline was included in this category, despite the fact that its use is by no means specific for AL amyloidosis. The differences seen between studies most likely relate to methodology and relatively small numbers of events. The studies with the most epidemiologically sound designs yielded very similar results[14 20 18 19].

[H2] *Prevalence*

The prevalence rates of systemic AL amyloidosis have increased due to improved therapies and overall survival of patients. Indeed, prevalence estimates were between 8.8 and 15.5 per million person-years before 2010 [15,17] but have since increased to 40-58 patients per million person-years (Table 2)[15, 18,17]. One study calculated an annual percentage change of 12% between 2007 and 2015 in the United States[17]. This annual percentage change existed for both males (11.5%) and females (12.3%)[17].

[H1] Mechanisms/pathophysiology

[H2] *Amyloid fibril formation*

As previously mentioned, the process underlying amyloidosis is the conversion of globular, soluble proteins into insoluble amyloid fibrils that deposit in vital organs and damage their function[1]. This complex process can be favored by several factors, such as mutations that destabilize the native protein structure and expose hydrophobic and protease-sensitive regions, increased protein concentrations, due to either greater protein synthesis or reduced clearance, or the intrinsic propensity of certain proteins to form amyloid fibrils that becomes apparent with aging. Typically, protein aggregation is countered by protein homeostasis (proteostasis), that functions to maintain the proteome, both intracellularly and extracellularly, in a native conformation, in the correct location and at the right concentration[21, 22]. Overall, ~ 1,600 molecules have a role in proteostasis, the efficiency of which declines with age[23]. When intracellular and/or extracellular proteostasis fail, protein aggregation might occur. Proteins with diverse structures and functions aggregate to form amyloid fibrils which have a highly ordered cross-β fibre structures and are characterized by antiparallel β-strands that are arranged perpendicular to the fibre, as demonstrated by x-ray diffraction[24]. Amyloid fibrils have a distinct diameter of 7.5–10.0 nm as determined using electron microscopy[25].

[H3] *AL amyloidosis fibril formation.*

AL amyloidosis is usually caused by the low-level expansion of an indolent B cell clone[26] that produces an immunoglobulin light chain λ (referred to as light chain in this Primer) in 75–80% of cases and κ light chains in the remaining cases (Figure 1). A high frequency (~40–60%) of the chromosomal translocation t(11;14), which juxtaposes the

immunoglobulin heavy chain locus (IgH) to the oncogene cyclin D1, characterizes this amyloid B cell clone[27]. Somatic mutations in *IGLV* (encoding the light chain variable region) reduce the fold stability of the native protein and increase protein dynamics, which favors endoproteolysis and the production of variable light chain domains that can cause amyloidosis[28]. Indeed, amyloidogenic light chains have a low fold stability and high protein dynamics compared with the light chains produced in multiple myeloma [29, 30]. In proteostasis, extracellular chaperones favor appropriate light chain folding and inhibit protein aggregation[31]. The aggregation of amyloidogenic light chains can occur owing to disruption to, or overwhelming of extracellular proteostasis (Figure 1). Other factors can facilitate protein aggregation and oligomer formation, such as the interactions of amyloidogenic light chains with the tissue microenvironment, such as with extracellular matrix components (including glycosaminoglycans, collagen and lipids)[32], shear forces, proteases, and metals (copper in particular)[33]. In addition, cell membrane surfaces have been hypothesised to facilitate fibril attachment, by acting as anchors for a cell-mediated seeding mechanism[34]. Once formed, oligomers of light chains are on the pathway to form highly organized amyloid fibrils. The pentraxin serum amyloid P component (SAP) is a circulating plasma protein that is universally present in amyloid deposits owing to its calcium-dependent binding to amyloid fibrils[35]. SAP has been reported to protect amyloid fibrils from degradation[36], making this protein an excellent candidate for amyloid scintigraphy and as a target for amyloid-directed immunotherapy. The accumulation of amyloid deposits in parenchymal tissue leads to tissue damage, which causes dysfunction of vital organs[2]. In addition, amyloid fibrils cause cytotoxicity and promote the misfolding of light chains and further oligomer formation[34]. Soluble prefibrillar species, mainly oligomers, also contribute to organ damage through proteotoxicity, increased cellular oxidative stress resulting in mitochondrial damage and reduced cell viability[37-39] (Figure 1).

The kinetics of fibril formation offers important clues for clinical management (Figure 2). The formation of amyloid fibrils begins from a solution of the monomeric native protein, which might misfold and assume an amyloidogenic, partially folded conformation. When the amount of partially folded proteins reaches a specific concentration, a critical fibrillar nucleus forms, which catalyses protein aggregation and fibril development. The critical concentration required for nucleation varies, and can be very low for very unstable light chains or high for more stable light chains. Initially, conditions do not favor aggregation, which corresponds to the ‘lag phase’ that precedes fibril formation. The kinetics of

aggregation dramatically changes after formation of the fibrillar nuclei owing to their catalytic role. The concentration of partially folded proteins that is necessary to elongate the amyloid fibrils is 10-20 fold lower than the concentration required for forming the first fibrillar nucleus, depending on the protein species[40]. Thus, early diagnosis of AL amyloidosis and administration of a rapidly acting therapy that produces a swift and deep reduction of the amyloid precursor is critical to halt fibril growth and disease progression. Amyloid deposits are in general persistent and unusually resistant to degradation. However, slow natural clearance of amyloid deposits, by endogenous immunological mechanisms in which macrophages play an important role, does occur[41]. Clearance of amyloid deposits may contribute considerably to recovery of organ function[2].

[H2] *Organ involvement*

The heart and the kidneys are the two most frequently affected organs in systemic AL amyloidosis, although all organs can be involved, except the brain (Figure 3). The precise molecular mechanisms underlying amyloid organ targeting remain elusive. Several investigators have shown that certain structural features related to the light chain variable region gene and gene family confer a higher risk of involvement of specific organs, possibly through interactions with resident cells. For example, the germ line gene (unarranged DNA segments inherited through the germline, before it is modified by rearrangement and somatic hypermutation) *IGLV6-57* is more common in patients with AL systemic amyloidosis than in the normal B cell repertoire and is associated with renal involvement[42]. Mesangial cells of the kidney have a propensity to form amyloid fibrils when incubated with light chain derived from *IGLV6-57* [42]. Cardiac tropism has been related to the *IGLV1-44* germ line gene, which confers a 5-fold increase in the chance of dominant heart involvement[43], [44]. Although λ light chains are responsible of most cases of systemic AL amyloidosis, κ light chain of the *IGKV1-33* germ line preferentially targets the liver[44].

Cardiac involvement is a key determinant of patient survival, therefore several investigators have studied the mechanisms of cardiac damage caused by misfolded light chain[37-39]. Cardiac dysfunction can result from amyloid deposits causing widespread disruption of tissue architecture and from proteotoxicity of the light chains[7]. Other speculated mechanisms of organ dysfunction include the perturbation of cellular membranes by amyloid fibrils, cell toxicity owing to fibril growth and the formation of

soluble light chain oligomers by amyloid fibrils, although these mechanisms require further study. In addition, AL amyloid fibrils are cytotoxic at low concentrations, whereas soluble amyloid light chains induce apoptosis, suggesting that the mechanisms of cytotoxicity differ between soluble protein and amyloid fibrils[34]. Exposing cardiac cells to light chains purified from patients with cardiac AL amyloidosis led to the increased production of reactive oxygen species (ROS) compared with control LC proteins isolated from patients without cardiac involvement[33, 38]. Amyloidogenic light chains from patients with AL amyloid cardiomyopathy can induce p38 MAPK signaling resulting in increased production of ROS, impaired calcium homeostasis, cell dysfunction and eventually cell death in isolated adult cardiomyocytes[37]. This p38 MAPK pathway mediates also the transcription of type B natriuretic peptide (BNP), a serum biomarker of cardiac stretch and damage[45], supporting a possible connection between cardiotoxic effects of light chain with induced MAPK signaling and BNP. This pathogenetic link is the basis of the use of the serum biomarker NT-proBNP (the amino-terminal fragment of BNP) in the management of patients with AL, from early detection of cardiac involvement, to the risk classification and in monitoring cardiac response to therapy[46]

[H1] Diagnosis, screening and prevention

A diagnosis of amyloidosis should be considered in any patient presenting with heart failure with preserved ejection fraction, nephrotic range proteinuria, a mixed axonal demyelinating peripheral neuropathy with autonomic features or carpal tunnel syndrome, hepatomegaly without imaging abnormalities, or any patient with a monoclonal gammopathy or atypical multiple myeloma. Taste alterations are common [7, 47] (Figure 3). In any patient with these clinical signs and symptoms, at a minimum, immunofixation electrophoresis of the serum and urine and an immunoglobulin free light chain assay (which assesses the concentration of κ and λ free light chains and their ratio in the serum) should be carried out to assess for a precursor light chain protein. Where available, imaging with radio-iodinated SAP can identify amyloid deposits in individuals with these syndromes[48] but this test has limited availability and is limited to certain specialized amyloidosis treatment centers. Tissue biopsy and histopathological analysis to confirm diagnosis is warranted in patients with an immunoglobulin light chain abnormality (Figure 4). The ordered ultrastructure of amyloid fibres allows the regular intercalation of Congo red dye, which shows green birefringence under polarized light microscopy; the diagnosis

of AL amyloidosis requires this histological observation (Figure 5). Although the direct biopsy of an affected organ will yield the diagnosis, it is generally not necessary as less-invasive investigations such as the aspiration of subcutaneous fat, a bone marrow biopsy, or lip biopsy can lead to diagnosis in 50–85% of patients[49, 50]. When amyloid deposits are detected in biopsies, accurate identifying the precursor protein is crucial to guide treatment. This is feasible using immunohistochemistry, in highly specialized laboratories[51, 52], and using immune-electronmicroscopy[53]. However, a mass spectrometry-based analysis of the amyloid-containing tissues is now considered the best approach with a reported sensitivity of 88% and specificity of 96% higher than immunochemical techniques and does not require a large panel of antisera to identify non AL amyloidosis[54]. Although not widely available, reference laboratories exist that will unequivocally confirm the protein subunit composing the amyloid fibril. This is particularly important in the black population with its high prevalence of V122I mutant ATTR amyloidosis can clinically resemble AL amyloidosis[55].

[H3] *Differential diagnosis.*

Diagnosing AL amyloidosis based on the presence of a serum and/or urine light chain abnormality and Congo red-positive tissue is insufficient. As many as 23% of patients with wild-type ATTR cardiac amyloidosis have a clonal immunoglobulin abnormality, which could result in misdiagnosis and inappropriate administration of chemotherapy[56]. Nuclear scintigraphy using ^{99m}Tc-labeled pyrophosphate (PYP) or ^{99m}Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) can be useful in differentiating cardiac AL amyloidosis from ATTR type [57, 58]. The mechanism by which these bone tracers bind to amyloid deposits is unclear, but substantial cardiac uptake occurs in virtually all patients with ATTR amyloidosis and in only about 40% of those with cardiac AL type; among the latter one-quarter do demonstrate substantial ATTR-like grade 2 or 3 uptake, which is associated with poor outcome [59].

When an amyloid deposit is detected as AL using mass spectroscopy, systemic amyloidosis should be distinguished from localized disease, as this determines management strategies. Localized deposits of AL that do not require systemic chemotherapy can be observed in the bladder, larynx, stomach, colon, skin, eyelids, lung, and urinary tract. Only systemic amyloidosis requires systemic therapy, as localized

disease usually has a very good prognosis[4]. A thorough evaluation for other areas of organ dysfunction can usually distinguish systemic from localized AL amyloidosis.

[H2] *Screening and prevention*

A substantial delay to diagnosis until after advanced organ damage has already ensued is still common for AL amyloidosis and, although therapeutic advances have been made, this results in high rates of death due to cardiac involvement and progression to end-stage renal disease in the first few months after diagnosis[8, 60]. The clinical manifestations of systemic AL amyloidosis resemble symptoms of more common conditions found in elderly individuals, therefore, appropriate diagnostic testing is usually initiated only several months after the onset of symptoms. Indeed, AL amyloidosis was diagnosed >1 year after the onset of symptoms in 40% of patients in one study[61]. Delays in diagnosis of AL amyloidosis are also common in patients with diagnosed monoclonal gammopathy of undetermined significance (MGUS) despite the appearance of amyloid-related symptoms[62]. Indeed, a monoclonal component with increased free light chain ratio can be consistently detected in the sera of patients with MGUS who eventually develop AL amyloidosis at least 4 years before the diagnosis[63]. Thus, patients with asymptomatic monoclonal gammopathy, MGUS or smoldering multiple myeloma, with an abnormal free light chain ratio are at risk of developing AL amyloidosis, and should be the target of screening programs. The heart and / or the kidneys are involved in >95% of patients with systemic AL amyloidosis, and biomarkers with 100% sensitivity are available to detect the presence of cardiac and renal involvement. For example, increased levels of NT-proBNP in serum can detect cardiac involvement in systemic AL amyloidosis before symptoms manifest, with 100% diagnostic sensitivity[64, 65]. When glomerular filtration rate (a marker of kidney function) is preserved, albuminuria can detect renal involvement at earlier disease stages when progression to end-stage renal disease can be almost invariably prevented[60]. Accordingly, assessment of NT-proBNP levels and for albuminuria should be integrated into the regular follow-up panel of patients with MGUS and an abnormal free light chain ratio[66, 67]. More than 95% of patients with AL amyloidosis have elevated NT-proBNP or albuminuria, and this approach can lead to the detection of pre-symptomatic systemic AL amyloidosis, that can be effectively treated with very good outcomes[68].

[H2] *Patient risk stratification*

The survival of patients with systemic AL amyloidosis is heterogeneous, depending on the severity of cardiac dysfunction at the time of diagnosis. Patients with diagnosis late in the clinical course (when heart damage is often irreversible) have a median survival of 3-6 months[69], whereas patients without cardiac involvement can survive for many years even if they fail to respond to first-line therapy. Similarly, the early diagnosis and effective treatment of patients with renal involvement almost abolishes the risk of progression to end-stage kidney disease and dialysis, whereas late diagnosis during the advanced stages of disease is associated with a higher risk of progression, despite treatment[60]. This heterogeneity requires accurate prognostic stratification to establish the best therapeutic approach, balancing treatment intensity and rapidity of action with patient frailty. Moreover, patient stratification is necessary for comparing results of clinical trials.

The current staging systems for systemic AL amyloidosis are based on the levels of circulating markers of cardiac, renal and B cell clonal disease. One cardiac staging system is based on the levels of NT-proBNP and cardiac troponins and was devised by the Mayo Clinic and modified by European investigators to improve the discrimination of very high risk patients (Figure 6A)[69-72]. This cardiac staging system is the most widely used for clinical trial design and to determine patient management. This staging system was modified to include clonal burden, assessed by bone marrow plasma cell infiltration and dFLC (difference between involved and uninvolved circulating free light chain) concentration, that have independent ability to predict survival. Patients with AL amyloidosis and a bone marrow plasma cell infiltrate of >10% have poorer survival that is comparable to patients with concomitant overt multiple myeloma[73]. Individuals with a very low (<50 mg/L) dFLC level have a significantly better outcome irrespective of cardiac stage[74-76]. The Mayo Clinic group incorporated the dFLC level (with a cutoff value of 180 mg/L) in their revised staging system (Figure 6B)[77, 78]. A renal staging system predicting the progression to dialysis has also been proposed and validated by European investigators (Figure 6C)[60, 79] Although the severity of renal involvement does not directly affect survival, it impacts kidney survival, QOL and might reduce access to effective therapy. Other biomarkers have been shown to predict outcomes in systemic AL amyloidosis, but have not been integrated in staging systems so far. For instance, high levels of von Willebrand factor was found to be associated with early death.[80] More

recently, growth differentiation factor 15 emerged as a predictor of both survival and progression to dialysis.[81]

[H1] Management

The aims of therapy are rapid elimination of the amyloid precursor and enhanced reabsorption of amyloid deposits, to rapidly ameliorate cardiac function to improve patients' quality of life and enhance survival. The suppression of amyloid light chain synthesis is effectively achieved using chemotherapy (both conventional and high dose) in combination with peripheral blood autologous hematopoietic stem cell transplantation, and, more recently, with immunotherapy targeting the B cell clone. Immunotherapies promoting the reabsorption of amyloid deposits are in clinical development[7]. Ultimately, the types of therapy used depend on the patient's risk classification.

Changes in levels of dFLC, NT-proBNP, proteinuria or GFR are used to assess treatment efficacy; indeed, an international effort established and validated hematologic and organ response criteria in AL amyloidosis (Table 3). The aim of chemotherapy should be the rapid achievement of very low absolute values (rather than percent reductions) of dFLC, which are associated with improvements in organ dysfunction and prolonged survival[82, 83] Emerging data indicate that minimal residual disease (MRD) may be responsible for residual organ disease despite what is generally considered good-quality hematologic response. If the available preliminary data are confirmed, the coexistence of persistent organ dysfunction in the absence of other causes and MRD could prompt further chemotherapy to obtain MRD negativity and improving the likelihood of organ response.[84, 85]. Assessment of treatment response should be frequent, and should be carried out at least every 2 cycles of chemotherapy or 3 months after stem cell transplantation, and more frequently for patients with severe cardiac involvement. Patients failing to achieve a good response should be rapidly shifted to alternate rescue regimens. Organ response usually closely follows hematologic response, and can be assessed as early as 3 months after treatment initiation, but can continue to improve afterwards.

[H3] *Treatment of low risk patients.*

The goal of treatment for AL amyloidosis is targeting the underlying clonal plasma cell dyscrasia, aiming for rapid and deep hematologic responses. High-dose intravenous

melphalan conditioning followed by autologous peripheral blood stem cell transplantation (HDM/SCT) has been used as treatment for highly selected patients with AL amyloidosis since the first reports in the mid 1990's[86]. The results of studies of HDM/SCT at single center and multiple centers are reported in Supplementary Table 1. The risk of major complications, including death, during stem cell mobilization and collection is ~15%,[87] and early treatment-related mortality is 2-15% after transplantation;[88] appropriate patient selection is the key to reduction in morbidity and mortality. Eligibility criteria for HDM/SCT vary between centers, but broadly require a confirmed tissue diagnosis of amyloidosis, clear evidence of a clonal plasma or B cell dyscrasia, and adequate measures of performance status (a grade of 0–2 at the ECOG performance status), cardiac function (a left ventricular ejection fraction of >40%, cardiac biomarkers below the thresholds, New York Heart Association class of <3), pulmonary function (O_2 saturation >95% on room air), hepatic function (total bilirubin level <2 mg/dL) and hemodynamic stability (baseline systolic blood pressure >90 mm Hg). Patients on hemodialysis or peritoneal dialysis are not excluded at some centers if other eligibility criteria are met.[89]

Several studies have evaluated the efficacy and morbidity associated with SCT. One multicenter randomized controlled trial demonstrated similar clonal hematologic responses and superior overall survival with conventional chemotherapy using oral melphalan and dexamethasone compared to HDM/SCT. However, this trial had major limitations including a treatment-related mortality (TRM) of 24% in the SCT treatment arm and a small sample size, 20% were excluded in the SCT arm and 13 were unable to proceed to transplant.[90] However, a landmark analysis of surviving patients at 6 months failed to show superiority of overall survival in this randomized trial. Another report from the Center for International Blood and Marrow Transplant Research registry showed improvement in overall survival and a reduction in early mortality with excellent 5-year survival after HDM/SCT.[91] Dose adapted melphalan strategy, with dose reductions depending on renal, cardiac parameters and age, increases the potentially suitable patient population for SCT and can lead to prolonged survival especially if a hematologic CR is achieved [92] [93]. Lower doses of melphalan could reduce treatment related toxicity, but also lower hematologic responses.[94]

The largest experience with HDM/SCT for AL amyloidosis is from the Mayo Clinic and Boston University. In 421 patients treated with HDM/SCT at Boston University, TRM was 11% overall, and decreased to 6% in the last 5 years of the study[95]. Median event-

free survival (EFS) was 2.6 years, whereas overall survival was 6.3 years, and one year after treatment, 43% of evaluable patients achieved a complete hematologic response, and 78% experienced an organ response. For patients who achieved CR, the median EFS was 8.3 years and median overall survival was 13.2 years. 195 patients did not obtain CR, and of these patients, 52% had an organ response, the median EFS was 2 years and median overall survival was 5.9 years. 26% of the patients who did not achieve CR remained clinically stable at 5 years of follow-up. An expanded series of 647 patients from the same center demonstrated hematologic relapses in 38.5% of patients at a median of 4.32 years in patients who achieved CR [96]. In a series of 422 patients from the Mayo Clinic, TRM was 12% in patients treated before 2006 and 7% after 2006. Troponin T levels >0.06 ng/L and NT pro-BNP levels >5,000 pg/mL were associated with high TRM, whereas patients with both markers below the thresholds had a TRM of 1%[97].

The first report of any organ improvement with respect to renal response following HDM/SCT demonstrated [98] a renal response in 36% of patients 12 months after treatment[98]. This response was defined as a 50% reduction in 24-hour urinary protein excretion in the absence of a ≥25% reduction in creatinine clearance. Renal response rate was 71% in patients a complete hematologic response and 11% for those with persistence of the plasma cell dyscrasia. Since this initial report, improvements in quality of life,[99] hepatic responses[100] and cardiac responses[101, 102] after HDM/SCT have been reported. Similar to renal response, clinical responses in other organ systems are more evident in patients with hematologic responses and can take up to 6-12 months or longer to occur. Given the association between survival and organ responses with hematologic response after SCT, strategies to improve hematologic complete response rates after this procedure have been an important focus. These include induction therapy prior to HDM/SCT[103, 104], novel conditioning regimens[105] and consolidation therapy[106]. The role of induction therapy for bone marrow plasmacytosis of >10% remains controversial, however, is recommended by some clinicians[73, 107].

Hematologic relapses or progression after HDM/SCT occurs in 36-38% at a median of 2.0 to 4.3 years after treatment[96]. Late relapses >20 years after HDM/SCT have also been reported.[96] Others have reported an EFS of ~4 years in patients undergoing stem cell transplantation for AL amyloidosis, independent of hematologic response, and superior EFS in individuals achieving complete response at 1 year post-transplant.[108]

[H3] *Treatment of intermediate-risk and high-risk patients.*

Patients at intermediate risk (that is, those with stage II or IIIa disease, Figure 6a) or high-risk (stage IIIb, Figure 6a) have increasing treatment options; however, the benefit of treatment for high-risk patients is considerably less compared with other patients[109]. A small proportion of patients with intermediate risk can safely undergo upfront autologous peripheral blood stem cell transplantation (HDM/SCT) as they have partially preserved organ, particularly cardiac, function. However, the best-suited therapy for those with intermediate-risk disease is unclear and practice patterns vary between amyloidosis centers.

A major breakthrough for patients with systemic AL amyloidosis was the introduction of oral chemotherapy with melphalan and dexamethasone (MDex) (Supplementary Table 2)[110]; this treatment was the standard for patients not undergoing HDM/SCT for more than a decade. MDex is very well tolerated in intermediate risk patients and a hematologic response is reached in up to 76% of patients, with very good partial response or complete response in 60% of cases when full dose dexamethasone can be given[78]. Regimens using bortezomib (a proteasome inhibitor) are now considered the upfront standard of care in most patients with AL amyloidosis. In the largest retrospective study of first-line treatment with cyclophosphamide, bortezomib and dexamethasone (CyBorD), the overall hematologic response rate was 66% in patients with stage II or IIIa disease, with a very good partial response or complete response in 47% of patients[109]. In studies comparing bortezomib-based combinations with previous standards of care, MDex and cyclophosphamide / thalidomide / dexamethasone response rates were higher with bortezomib treatment when combined with alkylating agents and dexamethasone, although this did not translate into a survival advantage[111, 112]. In an international phase III study, bortezomib plus MDex demonstrated a higher hematologic response rate compared with MDex alone (81% vs. 57%, P=0.005)[113].

Based on these data, intermediate-risk patients who are not eligible for HDM/SCT, should be treated with bortezomib-based regimens provided they do not have contraindications, such as peripheral neuropathy or fibrotic lung disease. Clonal and patient characteristics should be considered when choosing the most appropriate combinations; for example, treatment with bortezomib plus MDex can overcome the effects of both gain 1q21 (which confers a poorer outcome with oral melphalan) and t(11;14) (which confers a poorer outcome with bortezomib)[114-117]. Cyclophosphamide and

higher doses of dexamethasone do not significantly improve response rates and survival of patients with AL amyloidosis receiving bortezomib[118]. Treatment with bortezomib plus dexamethasone alone or in combination with cyclophosphamide is preferred in patients with potentially reversible contraindications to ASCT as these treatments are stem cell sparing, as well as in patients with renal failure, in whom melphalan dose usually requires adjustments. Intermediate risk patients in whom bortezomib is contraindicated due to pre-existing peripheral neuropathy can be treated with MDex or immunomodulatory drugs (IMiDs) based combinations, whereas patients without substantial peripheral neuropathy can receive cyclophosphamide / thalidomide / dexamethasone. The hematologic response rate to cyclophosphamide / thalidomide / dexamethasone is 68-79%, and at least a very good partial response can be observed in 45% of patients[112, 119]. However, substantial toxicity has been reported with thalidomide in patients with AL amyloidosis[120, 121]. Combining lenalidomide and MDex for frontline therapy led to hematologic response rates between 38% and 68% and substantial myelosuppression[122-124]. Lenalidomide, cyclophosphamide and dexamethasone combinatorial therapy has also been used as frontline therapy and has hematologic response rates from 46% to 60%, with at least a very good partial response in 40%-43% of patients[125-127].

High-risk patients represent ~20% of all individuals with AL amyloidosis, and represent a challenge owing to advanced cardiac stage (IIIb) or severe heart failure (NYHA class III or IV). So far, no treatment regimen can significantly alter the course of the disease in these patients, with median survival not exceeding 7 months[128]. Nevertheless, the few patients (approximately 20%) who survive long enough (1-3 months) to take advantage of response to chemotherapy can enjoy prolonged survival[129]. High-risk patients can be treated with low-dose combinations of the drugs used in intermediate-risk subjects, with weekly dose escalation based on tolerability with close monitoring[130]. Although high risk patients are typically excluded from clinical trials, there is interest in whether therapies directed at the amyloid itself may offer better hope.

[H3] *Treatment of relapse.*

Patients with relapsed disease have a good prognosis, with remarkably longer survival than patients with refractory disease[131, 132]. A few studies report the rate of relapse after initial treatment. The Boston University group reported a 38.5% rate of relapse after complete response following stem cell transplant.[96] A study by the Mayo Clinic

investigators revealed hematologic relapse or progression in 36% of patients who underwent HDM/SCT.[131] The Pavia Group reported that 35% of patients who received non-transplant upfront therapy needed second-line therapy after a median follow-up of 41 months.[132] No consensus has been reached on the criteria to commence rescue therapy in patients with relapsing disease[133]. Cardiac progression as assessed by increase in NT-proBNP should not be awaited, because it is associated with shorter survival[132]. Patients with relapsed disease can be treated by repeating upfront therapy, if possible, although this is associated with shorter time to retreatment without reduction in overall survival compared to patients who are treated with a different regimen at relapse[134]. For patients who have relapsed after autologous stem cell transplantation, treatment using a proteasome inhibitor is indicated. If the patient maintains eligibility and stem cells are available, a second autologous stem cell transplant may also be considered[131]. Although immunomodulatory drugs are less often considered as the first choice for patients with newly-diagnosed disease, they are often the backbone of treatment of refractory patients (Supplementary Table 3 and Figure 7). Lenalidomide can overcome resistance to alkylating agents, proteasome inhibitors, and thalidomide inducing a hematologic response also in patients refractory to these agents[135-140]. However, lenalidomide can worsen renal failure in patients with substantial proteinuria[141]. Pomalidomide is one of the most powerful agents in refractory AL amyloidosis, being able to rescue patients refractory to alkylators, first-generation and second-generation proteasome inhibitors, and lenalidomide[142-144]. Hematologic response to pomalidomide is obtained rapidly, in a median time of 1–2 months, and is observed in 48–68% of patients (with a very good partial response or complete response in 18–30%)[142-144] with a manageable toxicity profile. Newer agents have also been evaluated in patients with relapsed or refractory disease. In a phase II trial, the oral proteasome inhibitor ixazomib induced hematologic response in 56% of 21 previously treated patients, with all the 5 patients who had not been previously exposed to bortezomib achieving at least VGPR, and is currently being tested in a randomized phase III trial in patients with relapsed and/or refractory disease (NCT01659658)[145]. The humanized anti-CD38 monoclonal antibody daratumumab, is one of the most promising new agents[146, 147] and is being moved to frontline therapy in clinical trials. A recently published series of previously treated individuals who received daratumumab reported a rapid (median 1 months) hematologic response in 76% of patients with 36% complete responses[148].

[H2] Amyloid Directed Immunotherapy

Although chemotherapy reduces the plasma cell burden and ultimately the production of the amyloidogenic light chain protein, this therapy does not degrade amyloid deposited in tissues, although amyloid does slowly resorb from the body once the amyloid precursor has been suppressed. To this end, three monoclonal antibodies have been developed to target existing amyloid deposits: NEOD001, 11-1F4 and anti-SAP antibody.

In a phase I–II study, patients with AL amyloidosis who had completed at least one previous anti-plasma cell-directed therapy, had a partial hematologic response or better, and persistent organ dysfunction received NEOD001, which targets amyloid fibrils[149]. No drug-related serious adverse events or discontinuations were observed among the 27 treated patients, and of the 14 patients who could undergo cardiac evaluation, 8 responded and 6 were stable, whereas of the 15 patients who could go renal evaluation, 9 responded and 6 were stable. However, despite these results, a phase III trial and a phase IIb placebo-controlled trial failed to confirm the positive effects of NEOD001 on cardiac involvement, and the development of this antibody was discontinued. This emphasizes the need of controlled studies based on robust endpoints to introduce novel therapies for AL amyloidosis.

The murine monoclonal antibody 11-1F4 recognizes an amyloid-associated conformational epitope in human light chain-related fibrils[150]. In studies of mice with human amyloidomas (soft tissue tumors of AL composition created in the hindquarters of mice) 11-1F4 induced a rapid reduction of the masses without toxicity[151]. In an open-label, dose-escalation, phase 1 trial, 11-1F4 was well tolerated by all treated patients without dose-limited adverse events and promising organ responses after completion of phase 1a. Overall, 27 patients were treated with 11-1F4 in this study (8 patients during phase 1a and 19 patients during phase 1b). Of the evaluable patients, 63% demonstrated an organ response after infusion of 11-1F4 in phase 1a, and 61%demonstrated an organ response in phase 1b, with a median time to response of 2 weeks after the start of treatment[152]. No grade 4 or 5 adverse events were reported, and further clinical trials of 11-1F4 are currently being planned.

A third antibody approach is potentially applicable to all types of amyloidosis targeted SAP. Depletion of circulating SAP using miridesap allows the subsequent administration of the humanized anti-SAP antibody, dezamizumab, which binds to residual SAP in amyloid deposits and induces a macrophage response that triggers their rapid removal[153]. In an

open label, dose–escalation phase 1 clinical trial (NCT01777243), 16 patients with AA (amyloidosis A; formerly known as secondary amyloidosis), AL and AApoAI (Amyloid ApoplipoproteinA1) amyloidosis received a single dose of antibody. Mild infusion reactions and rashes were observed in some patients whom received larger doses, but no serious adverse events were reported. ^{123}I-SAP scintigraphy confirmed amyloid removal from the liver, spleen and kidneys, which were associated with improvements in liver function[154]. Further evaluation of the safety, pharmacokinetics, and dose-response effects of up to three cycles of miridesap followed by updating the accrual to dezamizumab to 23 patients (NCT01777243), demonstrated good tolerability and progressive dose-related clearance of amyloid[153]. A phase 2 trial of monthly repeated treatments in patients with cardiac AL and ATTR amyloidosis is on-going (NCT03044353).

[H2] Supportive therapy

Therapy for patients with AL amyloidosis is not limited to treating the underlying clone, but also includes supportive therapy. These patients with AL amyloidosis typically have a large symptom burden (Figure 3) owing to their underlying amyloid induced organ dysfunction, producing poor functional status at baseline and making them more susceptible to chemotherapy induced toxicity.

[H3] *Cardiac disease.*

Patients with cardiac dysfunction due to AL amyloidosis should be managed differently from those with cardiac dysfunction caused by other factors. Patients with AL cardiomyopathy do not typically tolerate β-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, or angiotensin receptor blockers. The sinus tachycardia for many of these patients is physiological and necessary to maintain adequate cardiac output. Careful diuresis, avoiding over-diuresis, and avoidance of drugs that may reduce cardiac output are the best strategies for the treatment of cardiac dysfunction owing to AL amyloidosis. Loop diuretics are most commonly used, of which, torsemide has a better bioavailability than furosemide. Spironolactone and metolazone can be used as adjunctive diuretics. Superimposed nephrotic syndrome or autonomic dysfunction makes the management of amyloid cardiomyopathy even more challenging since both hamper diuresis. In patients with atrial fibrillation or flutter, amiodarone is the best tolerated drug. Atrial ablation and atrioventricular nodal ablation can also be of value[155]. Careful use of digoxin in patients with atrial fibrillation or flutter and low blood pressure should not be

discounted[156]. Ventricular arrhythmias, especially premature ventricular contractions, are common and the presence of couplets and complex arrhythmias are prognostic;[157] however, defibrillators are less effective in patients with AL amyloidosis in part because pulseless electrical activity is one of the more common pre-terminal events[158-161]. Patients on β-blockers might have increased risk of bradycardia owing to complete atrioventricular block, and in one series, the pre-cardiac arrest rhythm was bradycardia in all 8 patients, including a complete heart block in 6 patients[162]. Which patients with AL amyloidosis might benefit from cardiac defibrillations is unclear. Pacemakers can be useful with patients with chronotropic incompetence.

Doxycycline has demonstrated anti-amyloid activities in vitro and in vivo. Doxycycline interferes with amyloid formation in a mouse model of AL amyloidosis[163] and abrogates light chain toxicity in vitro[164]. Indeed, the addition of doxycycline to standard chemotherapy reduced early mortality in cardiac AL amyloidosis in a retrospective case matched study[165]. An international phase III trial is ongoing in newly diagnosed patients with advanced cardiac involvement comparing standard of care (that is, bortezomib-based therapies) versus standard of care plus doxycycline (NCT03474458).

Orthotopic heart transplantation might be used in selected patients.[166] [167] Key determinants for the best outcomes following transplantation include limiting candidates to those who have lower tumor burden, accepting candidates who have clinical organ involvement limited to the heart and administering chemotherapy that is effective against the clone. However, most patients do not satisfy these criteria. Many patients awaiting transplant do not survive long enough to receive an orthotopic heart. For patients who do receive transplantation, five-year overall survival ranges from 18% to 66%[168]. Some of the best results have been in patients who have ASCT after their cardiac transplant[169, 170], but with improving therapies directed at the plasma cell clone, one could consider other non-ASCT options.

[H3] *Renal disease.*

For AL nephrotic syndrome nephrologists might recommend angiotensin-converting-enzyme inhibitors based on data from patients with diabetic nephropathy. Whether these drugs provide benefit in some patients with AL amyloidosis is unknown, but it is clear that they might be harmful in patients with coexistent AL cardiomyopathy or autonomic dysfunction. For patients with very low serum albumin levels, diuretics alone might be

insufficient to diurese them; albumin diuresis can be helpful to return patients closer to their dry weight. Peritoneal dialysis and hemodialysis are options for patients who develop renal failure, but for patients with either coexisting cardiac or autonomic involvement, hemodialysis can be a challenge due to hypotension[171]. Renal transplantation is an option for some patients with AL amyloidosis. However, amyloidosis can occur in a transplanted kidney,[172, 173] but should be less of an issue with highly effective anti-plasma cell directed therapies. In one study, among 22 patients receiving a kidney transplant in the United Kingdom, no renal graft failures were reported at 4.8 years, 1-year overall survival was 95% and 5-year OS was 67%[172]. In a Mayo Clinic series, overall survival was 84% at 1 year and 76% at 5 years in 19 patients with AL amyloidosis who received kidney transplantation, and no graft failures were reported [173].

[H3] *Other symptoms.*

Autonomic neuropathy alone or with other organ involvement is very difficult to manage. In patients with autonomic neuropathy without cardiomyopathy and nephrotic syndrome, a high salt diet and fludrocortisone administration are useful adjuncts to manage hypotension as are 40 mmHg compression hose—either thigh high or waist high. Even among patients with cardiomyopathy, midodrine, mestinon or droxidopa (not fludrocortisone) might be required to maintain adequate blood pressure. Diarrhea due to either autonomic neuropathy or gastrointestinal amyloid deposits can be managed using anti-motility agents, bile acid binders, octreotide, and even central parenteral nutrition. Clinical improvement in peripheral neuropathy is rare with traditional chemotherapy, and it is infrequent even with high-dose chemo therapy. Several drugs might be useful for painful neuropathy, such as gabapentin, pregabalin and duloxetine, and topical therapies containing lidocaine, amitriptyline, and ketamine might also be beneficial. Collaborative symptom management with rehabilitation physicians and/or palliative medicine teams might also be of value.

[H1] Quality of life

QOL is deeply, strongly and broadly affected in patients with systemic AL amyloidosis owing to multiorgan involvement and treatment. However, a consistent and standardized measurement of QOL in AL amyloidosis is not available. QOL measures can predict several outcomes such as job loss, work productivity, health expenditures and even

mortality, and many different tools are available to assess QOL. Commonly used tools to evaluate QOL in the area of stem cell transplantation are EORTC QOL-30 (European Organization for Research and Treatment of Cancer) and FACT-BMT (Functional Assessment of Cancer Therapy-Bone Marrow Transplant scale) instruments. The medical outcomes study 36-item short form general health survey (that is, the SF-36) questionnaire is the most reliable, rigorously validated, and widely used patient-reported outcome measure. The SF-36 Health Survey is currently the most used generic patient reported outcome measure in studies of patients with AL amyloidosis, and early qualitative validation studies support its use in this population. It has been reported with consistent evidence of the psychometric properties of the SF-36 in both community-based and clinic-based samples of patients with AL amyloidosis[174]. Aside from the SF-36, other outcomes scales, such as the Hematology Patient Reported Symptom Screen can predict survival and assess QOL in patients with AL amyloidosis. This tool is composed of three questions about fatigue, pain, and QOL[175].

In one observational study[176], significant improvements in vitality, social functioning, role-emotional and mental health were demonstrated after HDM/SCT in patients with AL amyloidosis[176]. Lower pre-treatment SF-36 physical component scores were associated with a greater risk of mortality in patients receiving HDM/SCT or those who received non-SCT chemotherapy regiments and during follow-up periods[176]. An improvement in SF-36 scores after HDM/SCT was also demonstrated in another observational study[99]; mental component summary scores reached the population norm 1 year post-SCT and physical component summary score reached the population norm 2 years post-SCT. In addition, certain domains of SF-36 scores could be used to predict survival following HDM/SCT; higher physical function (PCS domain) score was associated with early post-SCT survival and higher vitality scores (MCS domain) were associated with late post-SCT survival beyond one year[99].

The use of QOL assessments at every physician visit or treatment might provide valuable insights for treating rare conditions like AL amyloidosis. The effect of systemic AL amyloidosis on the QOL of patient's caregivers has not been studied. Similarly, the financial implications of this multiorgan disease on patients and caregivers — although tremendous — are not well documented.

[H1] Outlook

Great advances have been made during the last decade in understanding the mechanisms of AL amyloidosis and in treatment, which have translated into better QOL and improved survival. However, less than one-quarter of patients achieve a complete and long-lasting hematologic remission and survive for more than 10 years[7, 47]. Production of AL amyloid light chain precursors by clonal plasma cells still cannot be adequately suppressed in most cases, and the function of vital organs impaired by amyloid improves in only one quarter of patients on an intention to treat basis[7]. Furthermore, ~20% of patients are diagnosed at a late stage when cardiac damage is very advanced and survival can be measured in weeks. At the present time, therapy directed towards the underlying clonal disease improves the outcome in approximately 30% of these patients, but anti-amyloid therapies might increase survival in additional patients. Improved awareness of AL amyloidosis and diagnostic methods to enable earlier diagnosis before cardiac damage has become irreversible is one of the major aims of current research.

In addition, searching for new drugs is ongoing. For instance, the proteotoxicity exerted by amyloid, misfolded, LCs, could be harnessed for therapy. The distinctive perinuclear distribution of mitochondria in plasma cells from patients with amyloidosis as compared with plasma cells from patients with multiple myeloma and individuals with MGUS is indicative of oxidative stress, which was further supported by the abundance of transcripts encoding organelle-resident redox sensors[177]. Moreover, the expression of amyloidogenic light chains in myeloma cells altered cell growth and proteostasis through proteotoxicity, and conferred sensitivity to bortezomib; accordingly, proteasome inhibitors are targeted therapy in AL amyloidosis, and might direct future anti-clone drug development[177]. Drugs targeting the ubiquitin/proteasome system are under development for multiple myeloma in order to overcome resistance to proteasome inhibitors[178] and preliminary data obtained in primary amyloidogenic plasma cells indicate potential activity for AL amyloidosis.

Researchers are focusing their efforts on investigation of the biological characteristics of amyloidogenic B-cell clones and on development of novel agents and their optimal use in combinations to provide high rates of eradication. Sensitive technologies based on mass spectrometry are being developed to detect trace amounts of the amyloid LC, along with next generation flow cytometry and sequencing for the detection of minimal residual disease. Complete eradication of the clonal disease is expected to be associated with higher recovery of organ dysfunction and long-lasting remission and might become the

next therapeutic goal. Other open questions are the heterogeneity of organ involvement in a single patient and the mechanisms underlying the vital organ dysfunction caused by amyloidosis, particularly in the heart. Pathogenetic mechanisms being studied include direct disruption by amyloid of the myocardial architecture, coronary vasculature and autonomic nerves and cytotoxicity of amyloid fibrils and their soluble precursors. Although some ancestral models of amyloidosis (such as in *C. elegans* and zebrafish) have been used to investigate the mechanism of amyloid cardiac damage, other animal models of AL amyloidosis are urgently needed. The development of novel sensitive biomarkers and imaging technologies, notably including tissue characterization with MRI, for the detection and quantification of organ involvement and damage are already facilitating earlier diagnosis and enhanced evaluation of the efficacy of new and existing therapies. The outcome of ongoing trials investigating passive immunotherapy aimed at accelerating removal of amyloid will shortly shed exciting new light on this field, and inform further research aimed at improving recovery of the function of hearts damaged by amyloid, which is a prerequisite to significantly improving the overall outlook of this increasingly treatable disease.

Competing financial interests

G.M. is on the advisory board for Janssen, Pfizer and Caelum, and received travel support from Prothena and Janssen.

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G.P. sits on the advisory board of Janssen, received honoraria from Prothena and receives honoraria from Sebia and travel support from Celgene.

P.N.H. receives an honoraria from GSK and Alnylam and is a director and stockholder of Pentraxin Therapeutics Ltd.

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Figure 1: Schematic pathways involved in AL amyloid fibril formation.

A| The usually small and indolent B-cell clone can produce immunoglobulin light chain λ in 70-80% of cases. Somatic mutations in the light chain variable region *IGLV*, cause low folding stability and increased protein dynamics, which favors protein misfolding and improper aggregation. In addition, interactions between the protein and the tissue microenvironment, including extracellular matrix components, shear forces, endoproteases and metals, favors protein aggregation and oligomer formation. Cells may promote the initial nucleation of the deposits through interaction of the amyloid protein with cell membranes. Oligomers and, probably, the misfolded protein exert toxic effects by impairing cell function and reducing cell viability in target organs, and can develop to highly organized cross-β amyloid fibrils. Serum amyloid P component (SAP) protects amyloid fibrils from degradation and is ubiquitously present in amyloid deposits. Glycosaminoglycans (GAGs) serve as scaffolds and facilitate fibril formation. The accumulation of amyloid deposits in parenchymal tissue leads to tissue damage, which causes dysfunction of vital organs, moreover amyloid fibrils can cause cell damage and catalyze oligomers formation.

B| Light chains derived from certain genes show propensity to target specific organs: lambda LV1-44 preferentially targets the heart, lambda LV6-57 the kidney, and kappa KV1-33 the liver.

Figure 2: Kinetics of fibril formation in vitro.

A specific local concentration of partially folded proteins is necessary for the formation of a critical fibrillar nucleus. The critical concentration for nucleation varies, depending on the stability of the light chains. During the lag phase, the conditions do not favor protein aggregation and fibrils are not formed. However, after the formation of the fibrillar nuclei, protein aggregation into cross β-sheet oligomers occurs, leading to fibril formation and elongation. The concentration of partially folded proteins necessary for fibril elongation is substantially lower than the concentration required for forming the first nuclei. (Courtesy of Vittorio Bellotti).

Figure 3: Organ involvement in systemic AL amyloidosis.

The symptoms of AL amyloidosis are variable and mimic symptoms observed in common conditions of elderly individuals, such as heart failure (fatigue) diabetes mellitus (proteinuria and peripheral neuropathy), therefore contributing to late diagnosis. The

presence of heart failure with preserved ejection fraction and thickened ventricular walls with low voltages identified using electrocardiography should raise the suspicion of cardiac amyloidosis. Kidney involvement is characterized by proteinuria and progressive renal failure and manifests as peripheral oedema. The involvement of the gastrointestinal tract results in malabsorption and weight loss that can be prominent in some patients, whereas involvement of the autonomic nervous system can cause invalidating postural hypotension. The presence of prototypic signs such as macroglossia (enlargement of the tongue) and periorbital purpura can immediately lead to the right diagnosis. However, such signs are uncommon and, more importantly, appear late in the course of the disease, frequently when the organ damage caused by amyloid is already irreversible.

Figure 4. Diagnostic algorithm for systemic AL amyloidosis

The presence of heart failure with preserved ejection fraction and/or proteinuria with progressive renal failure in a patient with a monoclonal protein should raise the suspicion of systemic AL amyloidosis. The involvement of the peripheral and autonomic nervous systems as well as hepatomegaly associated with malabsorption and weight loss should also trigger the diagnostic process. In patients with a monoclonal protein and abnormal free light chain ratio, the unexplained increase in NT-proBNP > 332 ng/L and/or the presence of albuminuria >0.5 g/day are diagnostic red flags. In presence of cardiac involvement, technetium-labeled bone scintigraphy tracers, such as ^{99m}Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) and ^{99m}Tc-labeled pyrophosphate (PYP), help in distinguishing AL amyloidosis from ATTR amyloidosis. In patients without serum and urinary monoclonal protein a positive scan (≥ grade 2) is indicative of transthyretin amyloidosis. In patients with a monoclonal protein the biopsy of abdominal fat and lip salivary glands presents a 85% sensitivity for the diagnosis of AL amyloidosis. In patients with a negative biopsy who present with high index of suspicion of heart involvement, according to symptoms, echocardiography and ECG, cardiac MRI should be used promptly. If positive, cardiac biopsy and possibly amyloid typing is recommended. *Type amyloid with mass spectrometry or immunohistochemistry by very expert amyloid pathologist.

Figure 5: Histological evidence of amyloid fibrils in tissue.

Amyloid deposits are identified in tissue samples using Congo red staining and the detection of birefringence using polarized light microscopy. Abdominal fat aspirate from a patient with cardiac ATTR amyloid , who had both the TTR variant Val122Ile and an IgG kappa MGUS, shows uptake of Congo red (panel A) and birefringence under polarized light (panel B) and appears in bright-red in fluorescent light (excitation 497 nm and emission 614 nm). Renal tissue from a patient with light chain amyloidosis shows amyloid deposits in the glomeruli in bright light (panel D), in polarized light (panel E) and in fluorescent light (panel F). Cardiac tissue from a patient with light chain amyloidosis shows extensive amyloid deposition with vessel involvement in bright light (panel G), in polarized light (panel H) and in fluorescent light (panel I).

Figure 6: Risk Stratification of patients with AL amyloidosis.

Survival of 1065 patients diagnosed at the Pavia Amyloidosis Research and Treatment Center according to staging systems for survival and progression to dialysis.

A. This cardiac staging system is based on levels of the amino-terminal fragment of type B natriuretic peptide (NT-proBNP; with a cutoff level of 332 ng/L) and troponin I (cutoff level of 0.1 ng/mL). Patients are classified as stage I, II or III based on the presence of 0, 1, or 2 markers above the cutoff values, respectively. Troponin T can be used in the system instead of troponin I with a cutoff level of 0.06 ng/mL for standard tests and at 54 ng/L with high-sensitivity assays. Very high levels of NT-proBNP (>8,500 ng/L) identifies patients with advanced cardiac dysfunction (Stage IIIb) whereas stage III patients whose NT-proBNP is <8,500 ng/L have a better outcome (stage IIIa).

B. The Revised Mayo Clinic Staging system is based on NT-proBNP levels (cutoff 1,800 ng/L), troponin I levels (cutoff 0.07 ng/mL), and the difference between involved and uninvolved circulating free light chain (dFLC; cutoff 180 mg/L). Patients are classified as stage I, II, III or IV patients based on the presence of 0, 1, 2, or 3 markers above the cutoffs, respectively. Standard troponin T can be used in the system, instead of troponin I, with a cutoff at 0.035 ng/mL.

C. The renal staging system is based on proteinuria and estimated glomerular filtration rate (eGFR). The percentage/numbers for risk of dialysis for renal stage I, II and III may be different according to the treatment modality used. Stage I disease is based on the presence of both proteinuria < 5g/24h and eGFR >50 mL/min per 1.73 m2; Stage II is

based on either proteinuria >5g/24h or eGFR <50 mL/min per 1.73 m2; whereas stage III disease is based on both proteinuria >5g/24h and eGFR <50 mL/min per 1.73 m2
In all three staging systems, higher disease stages convey a higher risk of end-stage renal disease.

Figure 7. Risk-adapted treatment of AL amyloidosis.

Patients at low-risk (representing 20-25% of patients with AL amyloidosis) should receive full dose melphalan followed by autologous peripheral blood stem cell transplantation (HDM/SCT). Induction therapy with cyclophosphamide, bortezomib and dexamethasone (CyBorD) might be used in patients with bone marrow plasma cells (BMPC) >10%, and in patients who refuse upfront HDM/SCT. If less than a complete response (CR) is achieved 3 months after HDM/SCT, consolidation therapy with bortezomib and dexamethasone (BDex) should be considered. Patients at intermediate risk (representing ~60% of patients with AL amyloidosis) are those who are ineligible for HDM/SCT and without severe cardiac involvement. The combination of bortezomib, melphalan and dexamethasone (BMDex) can be used to treat patients with the common chromosomal translocation t(11;14), which confers a poor response to bortezomib, but these patients are sensitive to standard dose melphalan, and can be used in patients with 1q21, whom are poorly responsive to standard dose melphalan but who are sensitive to bortezomib. However, melphalan can impair stem cell collection in patients who are potential candidates for ASCT - CyBorD should be preferred in these patients. Patients presenting with peripheral neuropathy or fibrotic lung disease should avoid bortezomib due to its potential neurotoxicity and lung toxicity. Patients with severe cardiac involvement (20-25% of patients with AL amyloidosis), with very high serum concentration of NT-proBNP (>8500 ng/L) and NYHA class ≥ III are considered high-risk, represent an unmet need. Bortezomib-based regimens, either attenuated or full dose under close observation in a critical care unit, can benefit 30-40% of these patients, although the overall survival is poor (4-7 months). In these patients, cardiac transplantation should be considered, followed by ASCT. The treatment of relapsing/refractory patients is mostly based on immune-modulatory drugs (IMiDs), with pomalidomide emerging as well tolerated and fast acting. Daratumumab is highly effective, and based on the outcome of ongoing phase III trial, might be moved to upfront therapy in combination with bortezomib-containing regimes.

Table 1. Most common systemic amyloidoses

Designation**	Parent protein	Systemic and/ or localized	Acquired or hereditary	Organs involved
AL	Immunoglobulin light chain§	Systemic or Localized	Acquired (hereditary*)	Heart, kidney, liver, soft tissues, peripheral nervous system (including the autonomic nervous system) and gastrointestinal tract
ATTR	Transthyretin	Systemic	Hereditary	Peripheral nervous system (including the autonomic nervous system), heart, eye, kidney and leptomeninges
		Systemic	Acquired	Heart and ligaments
AA	Serum amyloid A protein	Systemic	Acquired	Predominantly kidney, but may involve liver, gastrointestinal tract and occasionally heart, thyroid, autonomic nervous system
ALECT2	Leukocyte chemotactic factor-2	Systemic	Acquired	Kidney, liver, spleen, adrenals and lungs
AApoAI	Apolipoprotein A I	Systemic	Hereditary	Heart, liver, kidney, peripheral nervous system, testis, larynx variants) and skin
AFib	Fibrinogen α	Systemic	Hereditary	Kidney, primarily, with obliterative glomerular involvement
Aβ2M	β2-Microglobulin, wild type	Systemic	Acquired (hemodialysis-related)	Musculoskeletal system
	β2-Microglobulin	Systemic	Hereditary	Autonomous nervous system

* One family with mutation in the constant region of κ light-chain, with cysteine replacing serine at amino acid residue 131 has been reported[179]

§ Rare cases of amyloidosis formed by immunoglobulin heavy chains (AH) and by heavy- and light-chains (AHL) have been reported.

The amyloid fibril protein is designated protein A and followed by a suffix that is an abbreviated form of the precursor protein name. For example, when amyloid (A) fibrils are derived from immunoglobulin light (L) chains the amyloid fibril protein is AL.

Table 2. Epidemiology Studies in AL amyloidosis

Annual incidence (per million person-years)	Annual prevalence (per million person-years)	Location (timeframe)	Study design	Median age (years)	% male patients	Reference
8.9 (5.1-12.8) *	No data	Olmsted county, Minnesota (1950-1990)	Population-based study; immunohistochemical typing for case ascertainment	73.5	62%	[14]
12 (95% CI 8, 16) *	No data	Olmsted county, Minnesota (1990-2015)	Population-based study; primarily mass spectrometry typing for case ascertainment (See above)	76	54%	[20]
12.5 (95% CI, 5.6-19.4) †	58 (95% CI, 43-73) †	Limousin region, France (2012 to 2016)	Population-based study; no mention of amyloid typing methodology	72.5	70%	[18]
3 ‡	Year 2000: 8.8 Year 2008: 20.4	England (2008)	Case ascertainment was extrapolated from death certificates and referral rates to and amyloidosis types at	Peaked at age 60-69	No data	[15]

			the National Amyloidosis Centre			
3.2 ‡	No data	Sweden (2001-2008)	Case ascertainment was extrapolated from myeloma statistics and amyloid hospital discharge diagnoses	No data	No data	[16]
6.2 (95% CI, 2.6-9.7)*,**	No data	Buenos Aires Argentina (2006-2015)	Case ascertainment extrapolated from registrants in the Medical Care Program in Buenos Aires	No data		[19]
10.8 -12.7 *	Year 2007: 15.5 Year 2015: 40.5	USA (2007-2015)	US claims data	64 #	54%	[17]

*Age and sex adjusted - † Crude estimate - ‡ Adjusted for age only - # Mean age - ** adjusted to the Buenos Aires Census (2010 census)

Table 3. Validated Treatment Response Criteria in AL Amyloidosis

Response	Definition of measurable disease	Criteria
Hematologic[74, 75, 82]	dFLC >50 mg/L	• Complete response: negative serum and urine immunofixation and normal FLC ratio • Very good partial response: dFLC <40 mg/L • Partial response: dFLC decrease >50% compared to baseline
	dFLC 20–50 mg/L	• low-dFLC response: dFLC <10 mg/L
Cardiac[82]	NT-proBNP > 650 ng/L	• NT-proBNP decrease >30% and >300 ng/L compared to baseline
Renal[60]	proteinuria >0.5 g/24h, predominantly albumin	• proteinuria decrease >30% compared to baseline (or is < 0.5 g/24h), in the absence of reduction in eGFR by >25%

dFLC, difference between involved (amyloidogenic) and uninvolved free light chain; eGFR, estimated glomerular filtration rate; FLC, free light chain; NT-proBNP, amino-terminal pro-natriuretic peptide type-B;