

1

## 2 Systemic immunoglobulin light chain amyloidosis

3

4

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

7 1. Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San  
8 Matteo, Pavia, Italy

9 2. Department of Molecular Medicine, University of Pavia, Pavia, Italy

10 3. Division of Hematology, Department of Internal Medicine, Mayo Clinic, Rochester, MN,  
11 USA

12 4. Section of Hematology-Oncology and Amyloidosis Center, Boston University School of  
13 Medicine, Boston, MA, USA

14 5. Department of Internal Medicine V (Haematology, Oncology and Rheumatology),  
15 Amyloidosis Center, University Hospital Heidelberg, Heidelberg, Germany

16 6. National Amyloidosis Centre, University College London (Royal Free Campus), London,  
17 UK

18

19 Correspondence:

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

21

22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

## **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- $\beta$ -sheet quaternary structure<sup>1</sup>. 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<sup>2</sup>; 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  $\beta$ -amyloid in Alzheimer disease, leading to localized amyloidosis, and others accumulate throughout the tissues of the body (known as systemic amyloidosis)<sup>3</sup>. At least 17 proteins can cause systemic amyloidosis;

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

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

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

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

93 This primer focuses on systemic AL amyloidosis, highlighting the disease  
94 mechanisms and basis for effective treatment, owing to advance in deciphering the  
95 molecular mechanisms of this form of amyloidosis and in developing novel, effective  
96 therapies that have improved quality of life and survival<sup>7, 8</sup>.

97

## 98 [H1] Epidemiology

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

118

## 119 [H2] Incidence of AL amyloidosis

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

121 carried out in the Americas and Europe<sup>14,15, 16,17-20</sup> (Table 2). The first study was carried  
122 out using the Olmsted County Project in Minnesota, USA, and reported an overall sex-  
123 adjusted and age-adjusted rate of 8.9 cases per million person-years between 1950 and  
124 1989 and 10.5 cases of systemic AL amyloidosis per million person-years between 1970  
125 and 1989<sup>14</sup>. An update to this study that included patients from the same region between  
126 1990 and 2015, demonstrated an incidence of 12 cases per million per year, which did not  
127 significantly differ from that reported in the earlier study<sup>20</sup> (Kyle et al Accepted Mayo Clinic  
128 Proc.). The only other true population-based study of the incidence of systemic AL  
129 amyloidosis was carried out in the Limousin region of France from 2012 to 2016<sup>18</sup>. This  
130 study demonstrated a crude yearly incidence of 12.5 cases per million inhabitants over the  
131 5 year period studied. The four other studies were not true population-based studies, and  
132 ascertained the incidence based on death certificate reports and hospital discharges,  
133 among other methods<sup>15,17,16, 19</sup>. One study extrapolated the incidence from referral rates to  
134 the UK National Amyloidosis Centre, death certificates, and the distribution of types of  
135 systemic amyloidosis cases seen at the center<sup>15</sup> and estimated an incidence of at least 3  
136 cases per million person-years in England in 2008. A study in Sweden used myeloma  
137 statistics and amyloid hospital discharge diagnoses to derive an annual incidence of 3  
138 cases per million person-years between 2001 and 2018<sup>16</sup>. An incidence of 6.1 per million  
139 person-years adjusted for the population of Buenos Aires (2010 census) based on 12  
140 persons with AL amyloidosis<sup>19</sup>. These investigators designed a prospective cohort of all  
141 members of a prepaid HMO in Buenos Aires between 2006 and 2015. They calculated the  
142 number of incident cases of amyloidosis per one million person-years and adjusted it using  
143 the Buenos Aires Census of 2010. Lastly, another study estimated the incidence of AL  
144 amyloidosis using US claims data between 2007 and 2015<sup>17</sup>, and reported an age-  
145 adjusted and gender-adjusted incidence of 10.8-15.2 cases per million person-years.  
146 However, this estimate might be high as this study differentiated patients with AL  
147 amyloidosis from other forms of amyloidosis based on the receipt of 'AL amyloidosis  
148 defining therapies', which included therapies that are not specific for AL amyloidosis.  
149 Doxycycline was included in this category, despite the fact that its use is by no means  
150 specific for AL amyloidosis. The differences seen between studies most likely relate to  
151 methodology and relatively small numbers of events. The studies with the most  
152 epidemiologically sound designs yielded very similar results<sup>14 20 18 19</sup>.

153

## 154 [H2] *Prevalence*

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

161

## 162 [H1] *Mechanisms/pathophysiology*

### 163 [H2] *Amyloid fibril formation*

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

### 180 [H3] *AL amyloidosis fibril formation.*

181 AL amyloidosis is usually caused by the low-level expansion of an indolent B cell clone<sup>26</sup>  
182 that produces an immunoglobulin light chain  $\lambda$  (referred to as light chain in this Primer) in  
183 75–80% of cases and  $\kappa$  light chains in the remaining cases (Figure 1). A high frequency  
184 (~40–60%) of the chromosomal translocation t(11;14), which juxtaposes the

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

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

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

228

## 229 [H2] *Organ involvement*

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

244

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

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

267

## 268 **[H1] Diagnosis, screening and prevention**

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

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

298

### 299 *[H3] Differential diagnosis.*

300 Diagnosing AL amyloidosis based on the presence of a serum and/or urine light chain  
301 abnormality and Congo red-positive tissue is insufficient. As many as 23% of patients with  
302 wild-type ATTR cardiac amyloidosis have a clonal immunoglobulin abnormality, which  
303 could result in misdiagnosis and inappropriate administration of chemotherapy<sup>56</sup>. Nuclear  
304 scintigraphy using <sup>99m</sup>Tc-labeled pyrophosphate (PYP) or <sup>99m</sup>Tc-labeled 3,3-diphosphono-  
305 1,2-propanodicarboxylic acid (DPD) can be useful in differentiating cardiac AL amyloidosis  
306 from ATTR type <sup>57, 58</sup>. The mechanism by which these bone tracers bind to amyloid  
307 deposits is unclear, but substantial cardiac uptake occurs in virtually all patients with ATTR  
308 amyloidosis and in only about 40% of those with cardiac AL type; among the latter one-  
309 quarter do demonstrate substantial ATTR-like grade 2 or 3 uptake, which is associated  
310 with poor outcome <sup>59</sup>.

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

316 disease usually has a very good prognosis<sup>4</sup>. A thorough evaluation for other areas of  
317 organ dysfunction can usually distinguish systemic from localized AL amyloidosis.

318

## 319 **[H2] Screening and prevention**

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

347

## 348 [H2] *Patient risk stratification*

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

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

380 recently, growth differentiation factor 15 emerged as a predictor of both survival and  
381 progression to dialysis.<sup>81</sup>

382

### 383 [H1] Management

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

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

408

### 409 [H3] *Treatment of low risk patients.*

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

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

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

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

445 free survival (EFS) was 2.6 years, whereas overall survival was 6.3 years, and one year  
446 after treatment, 43% of evaluable patients achieved a complete hematologic response,  
447 and 78% experienced an organ response. For patients who achieved CR, the median EFS  
448 was 8.3 years and median overall survival was 13.2 years. 195 patients did not obtain CR,  
449 and of these patients, 52% had an organ response, the median EFS was 2 years and  
450 median overall survival was 5.9 years. 26% of the patients who did not achieve CR  
451 remained clinically stable at 5 years of follow-up. An expanded series of 647 patients from  
452 the same center demonstrated hematologic relapses in 38.5% of patients at a median of  
453 4.32 years in patients who achieved CR<sup>96</sup>. In a series of 422 patients from the Mayo  
454 Clinic, TRM was 12% in patients treated before 2006 and 7% after 2006. Troponin T levels  
455 >0.06 ng/L and NT pro-BNP levels >5,000 pg/mL were associated with high TRM,  
456 whereas patients with both markers below the thresholds had a TRM of 1%<sup>97</sup>.

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

472 Hematologic relapses or progression after HDM/SCT occurs in 36-38% at a median  
473 of 2.0 to 4.3 years after treatment<sup>96</sup>. Late relapses >20 years after HDM/SCT have also  
474 been reported.<sup>96</sup> Others have reported an EFS of ~4 years in patients undergoing stem  
475 cell transplantation for AL amyloidosis, independent of hematologic response, and  
476 superior EFS in individuals achieving complete response at 1 year post-transplant.<sup>108</sup>  
477

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

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

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

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

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

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

538

### 539 [H3] *Treatment of relapse.*

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

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

## 578 [H2] Amyloid Directed Immunotherapy

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

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

595 The murine monoclonal antibody 11-1F4 recognizes an amyloid-associated  
596 conformational epitope in human light chain-related fibrils<sup>150</sup>. In studies of mice with human  
597 amyloidomas (soft tissue tumors of AL composition created in the hindquarters of mice)  
598 11-1F4 induced a rapid reduction of the masses without toxicity<sup>151</sup>. In an open-label, dose-  
599 escalation, phase 1 trial, 11-1F4 was well tolerated by all treated patients without dose-  
600 limited adverse events and promising organ responses after completion of phase 1a.  
601 Overall, 27 patients were treated with 11-1F4 in this study (8 patients during phase 1a and  
602 19 patients during phase 1b). Of the evaluable patients, 63% demonstrated an organ  
603 response after infusion of 11-1F4 in phase 1a, and 61% demonstrated an organ response  
604 in phase 1b, with a median time to response of 2 weeks after the start of treatment<sup>152</sup>. No  
605 grade 4 or 5 adverse events were reported, and further clinical trials of 11-1F4 are  
606 currently being planned.

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

611 open label, dose–escalation phase 1 clinical trial (NCT01777243), 16 patients with AA  
612 (amyloidosis A; formerly known as secondary amyloidosis), AL and AApoAI (Amyloid  
613 ApolipoproteinA1) amyloidosis received a single dose of antibody. Mild infusion reactions  
614 and rashes were observed in some patients whom received larger doses, but no serious  
615 adverse events were reported. <sup>123</sup>I-SAP scintigraphy confirmed amyloid removal from the  
616 liver, spleen and kidneys, which were associated with improvements in liver function<sup>154</sup>.  
617 Further evaluation of the safety, pharmacokinetics, and dose-response effects of up to  
618 three cycles of miridesap followed by updating the accrual to dezamizumab to 23 patients  
619 (NCT01777243), demonstrated good tolerability and progressive dose-related clearance of  
620 amyloid<sup>153</sup>. A phase 2 trial of monthly repeated treatments in patients with cardiac AL and  
621 ATTR amyloidosis is on-going (NCT03044353).

622

## 623 [H2] Supportive therapy

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

629

## 630 [H3] Cardiac disease.

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

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

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

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

671

### 672 [H3] *Renal disease.*

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

678 insufficient to diurese them; albumin diuresis can be helpful to return patients closer to  
679 their dry weight. Peritoneal dialysis and hemodialysis are options for patients who develop  
680 renal failure, but for patients with either coexisting cardiac or autonomic involvement,  
681 hemodialysis can be a challenge due to hypotension<sup>171</sup>. Renal transplantation is an option  
682 for some patients with AL amyloidosis. However, amyloidosis can occur in a transplanted  
683 kidney,<sup>172, 173</sup> but should be less of an issue with highly effective anti-plasma cell directed  
684 therapies. In one study, among 22 patients receiving a kidney transplant in the United  
685 Kingdom, no renal graft failures were reported at 4.8 years, 1-year overall survival was  
686 95% and 5-year OS was 67%<sup>172</sup>. In a Mayo Clinic series, overall survival was 84% at 1  
687 year and 76% at 5 years in 19 patients with AL amyloidosis who received kidney  
688 transplantation, and no graft failures were reported <sup>173</sup>.

### 689 [H3] *Other symptoms.*

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

704

## 705 [H1] **Quality of life**

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

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

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

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

741

## 742 [H1] Outlook

743 Great advances have been made during the last decade in understanding the  
744 mechanisms of AL amyloidosis and in treatment, which have translated into better QOL  
745 and improved survival. However, less than one-quarter of patients achieve a complete and  
746 long-lasting hematologic remission and survive for more than 10 years<sup>7, 47</sup>. Production of  
747 AL amyloid light chain precursors by clonal plasma cells still cannot be adequately  
748 suppressed in most cases, and the function of vital organs impaired by amyloid improves  
749 in only one quarter of patients on an intention to treat basis<sup>7</sup>. Furthermore, ~20% of  
750 patients are diagnosed at a late stage when cardiac damage is very advanced and  
751 survival can be measured in weeks. At the present time, therapy directed towards the  
752 underlying clonal disease improves the outcome in approximately 30% of these patients,  
753 but anti-amyloid therapies might increase survival in additional patients. Improved  
754 awareness of AL amyloidosis and diagnostic methods to enable earlier diagnosis before  
755 cardiac damage has become irreversible is one of the major aims of current research.  
756 In addition, searching for new drugs is ongoing. For instance, the proteotoxicity exerted by  
757 amyloid, misfolded, LCs, could be harnessed for therapy. The distinctive perinuclear  
758 distribution of mitochondria in plasma cells from patients with amyloidosis as compared  
759 with plasma cells from patients with multiple myeloma and individuals with MGUS is  
760 indicative of oxidative stress, which was further supported by the abundance of transcripts  
761 encoding organelle-resident redox sensors<sup>177</sup>. Moreover, the expression of amyloidogenic  
762 light chains in myeloma cells altered cell growth and proteostasis through proteotoxicity,  
763 and conferred sensitivity to bortezomib; accordingly, proteasome inhibitors are targeted  
764 therapy in AL amyloidosis, and might direct future anti-clone drug development<sup>177</sup>. Drugs  
765 targeting the ubiquitin/proteasome system are under development for multiple myeloma in  
766 order to overcome resistance to proteasome inhibitors<sup>178</sup> and preliminary data obtained in  
767 primary amyloidogenic plasma cells indicate potential activity for AL amyloidosis.  
768 Researchers are focusing their efforts on investigation of the biological characteristics of  
769 amyloidogenic B-cell clones and on development of novel agents and their optimal use in  
770 combinations to provide high rates of eradication. Sensitive technologies based on mass  
771 spectrometry are being developed to detect trace amounts of the amyloid LC, along with  
772 next generation flow cytometry and sequencing for the detection of minimal residual  
773 disease. Complete eradication of the clonal disease is expected to be associated with  
774 higher recovery of organ dysfunction and long-lasting remission and might become the

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

791

#### 792 **Competing financial interests**

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

795 A.D. receives research support from Takeda, Pfizer, Celgene, Alnylam, GSK and received  
796 research support from Prothena.

797 V.S.: sits on the advisory boards of Janssen and Caelum and receives research support  
798 from Celgene, Takeda, Janssen and received research support from Prothena.

799 S.O.S. received honoraria and research support from Prothena, and receives honoraria  
800 from Janssen, travel support from Takeda and Jazz and research support from Janssen  
801 and Sanofi.

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

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

806 M.A.G. receives honoraria from Ionis, Abbvie, Alnylam, Amgen, Annexon, Appellis,

807 Celgene, Janssen, Johnson and Johnson, Medscape, Physicians Education Resource,

808 Prothena, Research to Practice, Spectrum and Teva, and receives research support from  
809 Spectrum.

810

### 811 **Acknowledgments**

812 G.M. and G.P. are supported in part by grants from “Associazione Italiana per la Ricerca  
813 sul Cancro–Special Program Molecular Clinical Oncology 5 per mille n. 9965,” from  
814 CARIPLO “Structure-function relation of amyloid: understanding the molecular bases of  
815 protein misfolding diseases to design new treatments n. 2013-0964,” and from CARIPLO  
816 “Molecular mechanisms of immunoglobulin toxicity in age-related plasma cell dyscrasias n.  
817 2015-0591.” G.P. is supported in part by the Bart Barlogie Young Investigator Award from  
818 the International Myeloma Society.

819

### References

820

- 821 1. Merlini, G. & Bellotti, V. Molecular mechanisms of amyloidosis. *N Engl J Med* **349**,  
822 583-96 (2003).
- 823 2. Pepys, M.B. Amyloidosis. *Annu Rev Med* **57**, 223-41 (2006).
- 824 3. Sipe, J.D. et al. Amyloid fibril proteins and amyloidosis: chemical identification and  
825 clinical classification International Society of Amyloidosis 2016 Nomenclature  
826 Guidelines. *Amyloid* **23**, 209-213 (2016).
- 827 4. Kourelis, T.V. et al. Presentation and Outcomes of Localized Immunoglobulin Light  
828 Chain Amyloidosis: The Mayo Clinic Experience. *Mayo Clin Proc* **92**, 908-917  
829 (2017).
- 830 5. Obici, L. & Merlini, G. Amyloidosis in autoinflammatory syndromes. *Autoimmun Rev*  
831 **12**, 14-7 (2012).
- 832 6. Nasr, S.H., Dogan, A. & Larsen, C.P. Leukocyte Cell-Derived Chemotaxin 2-  
833 Associated Amyloidosis: A Recently Recognized Disease with Distinct  
834 Clinicopathologic Characteristics. *Clin J Am Soc Nephrol* **10**, 2084-93 (2015).
- 835 7. Merlini, G. AL amyloidosis: from molecular mechanisms to targeted therapies.  
836 *Hematology Am Soc Hematol Educ Program* **2017**, 1-12 (2017).
- 837 8. Muchtar, E. et al. Improved outcomes for newly diagnosed AL amyloidosis between  
838 2000 and 2014: cracking the glass ceiling of early death. *Blood* **129**, 2111-2119  
839 (2017).

- 840 9. Quock, T.P., Yan, T., Chang, E., Guthrie, S. & Broder, M.S. Epidemiology of AL  
841 amyloidosis: a real-world study using US claims data. *Blood Adv* **2**, 1046-1053  
842 (2018).
- 843 10. Kyle, R.A. et al. Long-Term Follow-up of Monoclonal Gammopathy of Undetermined  
844 Significance. *N Engl J Med* **378**, 241-249 (2018).
- 845 11. Desikan, K.R. et al. Incidence and impact of light chain associated (AL) amyloidosis  
846 on the prognosis of patients with multiple myeloma treated with autologous  
847 transplantation. *Leukemia & Lymphoma* **27**, 315-319 (1997).
- 848 12. Madan, S. et al. Clinical features and treatment response of light chain (AL)  
849 amyloidosis diagnosed in patients with previous diagnosis of multiple myeloma.  
850 *Mayo Clin Proc* **85**, 232-8 (2010).
- 851 13. da Silva Filho, M.I. et al. Genome-wide association study of immunoglobulin light  
852 chain amyloidosis in three patient cohorts: comparison with myeloma. *Leukemia* **31**,  
853 1735-1742 (2017).
- 854 14. Kyle, R.A. et al. Incidence and natural history of primary systemic amyloidosis in  
855 Olmsted County, Minnesota, 1950 through 1989. *Blood* **79**, 1817-22 (1992).
- 856 15. Pinney, J.H. et al. Systemic amyloidosis in England: an epidemiological study. *Br J*  
857 *Haematol* **161**, 525-32 (2013).
- 858 16. Hemminki, K., Li, X., Forsti, A., Sundquist, J. & Sundquist, K. Incidence and survival  
859 in non-hereditary amyloidosis in Sweden. *BMC Public Health* **12**, 974 (2012).
- 860 17. Quock, T.P., Yan, T., Chang, E., Guthrie, S. & Broder, M.S. Healthcare resource  
861 utilization and costs in amyloid light-chain amyloidosis: a real-world study using US  
862 claims data. *J Comp Eff Res* (2018).
- 863 18. Duhamel, S. et al. Incidence and Prevalence of Light Chain Amyloidosis: A  
864 Population-Based Study. *Blood* **130**, 5577-5577 (2017).
- 865 19. Aguirre, M.A. et al. Incidence rate of amyloidosis in patients from a medical care  
866 program in Buenos Aires, Argentina: a prospective cohort. *Amyloid* **23**, 184-187  
867 (2016).
- 868 20. Kyle, R.A.e.a. Incidence of AL Amyloidosis in Olmsted County, Minnesota, 1990  
869 through 2015. *Submitted* (2018).
- 870 21. Hipp, M.S., Park, S.H. & Hartl, F.U. Proteostasis impairment in protein-misfolding  
871 and -aggregation diseases. *Trends Cell Biol* **24**, 506-14 (2014).

- 872 22. Yerbury, J.J. et al. Walking the tightrope: proteostasis and neurodegenerative  
873 disease. *J Neurochem* **137**, 489-505 (2016).
- 874 23. Labbadia, J. & Morimoto, R.I. The biology of proteostasis in aging and disease.  
875 *Annu Rev Biochem* **84**, 435-64 (2015).
- 876 24. Eanes, E.D. & Glenner, G.G. X-ray diffraction studies on amyloid filaments. **16**,  
877 673-677 (1968).
- 878 25. Shirahama, T. & Cohen, A.S. High-resolution electron microscopic analysis of the  
879 amyloid fibril. *J Cell Biol* **33**, 679-708 (1967).
- 880 26. Merlini, G. & Stone, M.J. Dangerous small B-cell clones. *Blood* **108**, 2520-30  
881 (2006).
- 882 27. Bochtler, T. et al. Hyperdiploidy is less frequent in AL amyloidosis compared with  
883 monoclonal gammopathy of undetermined significance and inversely associated  
884 with translocation t(11;14). *Blood* **117**, 3809-15 (2011).
- 885 28. Morgan, G.J. & Kelly, J.W. The kinetic stability of a full-length antibody light chain  
886 dimer determines whether endoproteolysis can release amyloidogenic variable  
887 domains. *J Mol Biol* **428**, 4280-4297 (2016).
- 888 29. Blancas-Mejia, L.M. et al. Thermodynamic and fibril formation studies of full length  
889 immunoglobulin light chain AL-09 and its germline protein using scan rate  
890 dependent thermal unfolding. *Biophys Chem* **207**, 13-20 (2015).
- 891 30. Oberti, L. et al. Concurrent structural and biophysical traits link with immunoglobulin  
892 light chains amyloid propensity. *Sci Rep* **7**, 16809 (2017).
- 893 31. Wyatt, A.R., Yerbury, J.J., Dabbs, R.A. & Wilson, M.R. Roles of extracellular  
894 chaperones in amyloidosis. *J Mol Biol* **421**, 499-516 (2012).
- 895 32. Ami, D. et al. In situ characterization of protein aggregates in human tissues  
896 affected by light chain amyloidosis: a FTIR microspectroscopy study. *Sci Rep* **6**,  
897 29096 (2016).
- 898 33. Diomedea, L. et al. Cardiac Light Chain Amyloidosis: The Role of Metal Ions in  
899 Oxidative Stress and Mitochondrial Damage. *Antioxid Redox Signal* **27**, 567-582  
900 (2017).
- 901 34. Marin-Argany, M. et al. Cell Damage in Light Chain Amyloidosis: Fibril  
902 internalization, toxicity and cell-mediated seeding *J Biol Chem* **291**, 19813-25  
903 (2016).

- 904 35. Pepys, M.B., Dyck, R.F., de Beer, F.C., Skinner, M. & Cohen, A.S. Binding of serum  
905 amyloid P-component (SAP) by amyloid fibrils. *Clin Exp Immunol* **38**, 284-93  
906 (1979).
- 907 36. Tennent, G.A., Lovat, L.B. & Pepys, M.B. Serum amyloid P component prevents  
908 proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis.  
909 *Proc Natl Acad Sci U S A* **92**, 4299-303 (1995).
- 910 37. Shi, J. et al. Amyloidogenic light chains induce cardiomyocyte contractile  
911 dysfunction and apoptosis via a non-canonical p38alpha MAPK pathway. *Proc Natl*  
912 *Acad Sci U S A* **107**, 4188-93 (2010).
- 913 38. Brenner, D.A. et al. Human amyloidogenic light chains directly impair cardiomyocyte  
914 function through an increase in cellular oxidant stress. *Circulation Research* **94**,  
915 1008-1010 (2004).
- 916 39. Imperlini, E. et al. Proteotoxicity in cardiac amyloidosis: amyloidogenic light chains  
917 affect the levels of intracellular proteins in human heart cells. *Sci Rep* **7**, 15661  
918 (2017).
- 919 40. Westermark, G.T., Fandrich, M., Lundmark, K. & Westermark, P. Noncerebral  
920 Amyloidoses: Aspects on Seeding, Cross-Seeding, and Transmission. *Cold Spring*  
921 *Harb Perspect Med* **8** (2018).
- 922 41. Nystrom, S.N. & Westermark, G.T. AA-Amyloid is cleared by endogenous  
923 immunological mechanisms. *Amyloid* **19**, 138-45 (2012).
- 924 42. Comenzo, R.L., Zhang, Y., Martinez, C., Osman, K. & Herrera, G.A. The tropism of  
925 organ involvement in primary systemic amyloidosis: contributions of Ig V-L germ  
926 line gene use and clonal plasma cell burden. *Blood* **98**, 714-720 (2001).
- 927 43. Perfetti, V. et al. The repertoire of lambda light chains causing predominant amyloid  
928 heart involvement and identification of a preferentially involved germline gene,  
929 IGLV1-44. *Blood* **119**, 144-50 (2012).
- 930 44. Kourelis, T.V. et al. Clarifying immunoglobulin gene usage in systemic and localized  
931 immunoglobulin light-chain amyloidosis by mass spectrometry. *Blood* **129**, 299-306  
932 (2017).
- 933 45. Ma, K.K., Ogawa, T. & de Bold, A.J. Selective upregulation of cardiac brain  
934 natriuretic peptide at the transcriptional and translational levels by pro-inflammatory  
935 cytokines and by conditioned medium derived from mixed lymphocyte reactions via  
936 p38 MAP kinase. *J Mol Cell Cardiol* **36**, 505-13 (2004).

- 937 46. Merlini, G. et al. Rationale, application and clinical qualification for NT-proBNP as a  
938 surrogate end point in pivotal clinical trials in patients with AL amyloidosis.  
939 *Leukemia* **30**, 1979-1986 (2016).
- 940 47. Wechalekar, A.D., Gillmore, J.D. & Hawkins, P.N. Systemic amyloidosis. *Lancet*  
941 **387**, 2641-54 (2016).
- 942 48. Lovat, L.B., Persey, M.R., Madhoo, S., Pepys, M.B. & Hawkins, P.N. The liver in  
943 systemic amyloidosis: insights from 123I serum amyloid P component scintigraphy  
944 in 484 patients. *Gut* **42**, 727-34 (1998).
- 945 49. Quarta, C.C. et al. Diagnostic sensitivity of abdominal fat aspiration in cardiac  
946 amyloidosis. *Eur Heart J* **38**, 1905-1908 (2017).
- 947 50. Muchtar, E. et al. Overuse of organ biopsies in immunoglobulin light chain  
948 amyloidosis (AL): the consequence of failure of early recognition. *Ann Med* **49**, 545-  
949 551 (2017).
- 950 51. Schonland, S.O. et al. Immunohistochemistry in the classification of systemic forms  
951 of amyloidosis: a systematic investigation of 117 patients. *Blood* **119**, 488-93  
952 (2012).
- 953 52. Linke, R.P. On typing amyloidosis using immunohistochemistry. Detailed  
954 illustrations, review and a note on mass spectrometry. *Prog Histochem Cytochem*  
955 **47**, 61-132 (2012).
- 956 53. Fernandez de Larrea, C. et al. A practical approach to the diagnosis of systemic  
957 amyloidoses. *Blood* **125**, 2239-44 (2015).
- 958 54. Vrana, J.A. et al. Clinical diagnosis and typing of systemic amyloidosis in  
959 subcutaneous fat aspirates by mass spectrometry-based proteomics.  
960 *Haematologica* **99**, 1239-47 (2014).
- 961 55. Pont, L. et al. A chemometric approach for characterization of serum transthyretin in  
962 familial amyloidotic polyneuropathy type I (FAP-I) by electrospray ionization-ion  
963 mobility mass spectrometry. *Talanta* **181**, 87-94 (2018).
- 964 56. Geller, H.I. et al. Prevalence of Monoclonal Gammopathy in Wild-Type  
965 Transthyretin Amyloidosis. *Mayo Clin Proc* **92**, 1800-1805 (2017).
- 966 57. Aljaroudi, W.A. et al. Role of imaging in the diagnosis and management of patients  
967 with cardiac amyloidosis: state of the art review and focus on emerging nuclear  
968 techniques. *J Nucl Cardiol* **21**, 271-83 (2014).

- 969 58. Gillmore, J.D. et al. Nonbiopsy Diagnosis of Cardiac Transthyretin Amyloidosis.  
970 *Circulation* **133**, 2404-12 (2016).
- 971 59. Zheng, J. et al. 99mTc-DPD scintigraphy is an independent predictor of outcomes in  
972 cardiac AL amyloidosis. *ISA 2018 Abstract Book*, OP46 page 107 (2018).
- 973 60. Palladini, G. et al. A staging system for renal outcome and early markers of renal  
974 response to chemotherapy in AL amyloidosis. *Blood* **124**, 2325-2332 (2014).
- 975 61. Lousada, I., Comenzo, R.L., Landau, H., Guthrie, S. & Merlini, G. Light Chain  
976 Amyloidosis: Patient Experience Survey from the Amyloidosis Research  
977 Consortium. *Adv Ther* **32**, 920-8 (2015).
- 978 62. Kourelis, T.V. et al. Immunoglobulin light chain amyloidosis is diagnosed late in  
979 patients with preexisting plasma cell dyscrasias. *Am J Hematol* **89**, 1051-4 (2014).
- 980 63. Weiss, B.M. et al. Increased serum free light chains precede the presentation of  
981 immunoglobulin light chain amyloidosis. *J Clin Oncol* **32**, 2699-704 (2014).
- 982 64. Palladini, G. et al. Serum N-terminal pro-brain natriuretic peptide is a sensitive  
983 marker of myocardial dysfunction in AL amyloidosis. *Circulation* **107**, 2440-5 (2003).
- 984 65. Wechalekar, A.D. et al. Abnormal N-terminal fragment of brain natriuretic peptide in  
985 patients with light chain amyloidosis without cardiac involvement at presentation is a  
986 risk factor for development of cardiac amyloidosis. *Haematologica* **96**, 1079-80  
987 (2011).
- 988 66. Merlini, G. & Palladini, G. Differential diagnosis of monoclonal gammopathy of  
989 undetermined significance. *Hematology Am Soc Hematol Educ Program* **2012**, 595-  
990 603 (2012).
- 991 67. Merlini, G., Wechalekar, A.D. & Palladini, G. Systemic light chain amyloidosis: an  
992 update for treating physicians. *Blood* **121**, 5124 - 5130 (2013).
- 993 68. Palladini, G. et al. Biomarker-based screening of organ dysfunction in patients with  
994 MGUS allows early diagnosis of AL amyloidosis. *Blood* **130**, 1670 [abstract] (2017).
- 995 69. Wechalekar, A.D. et al. A European collaborative study of treatment outcomes in  
996 346 patients with cardiac stage III AL amyloidosis. *Blood* **121**, 3420-3427 (2013).
- 997 70. Dispenzieri, A. et al. Serum cardiac troponins and N-terminal pro-brain natriuretic  
998 peptide: a staging system for primary systemic amyloidosis. *J Clin Oncol* **22**, 3751-7  
999 (2004).

- 1000 71. Kristen, A.V. et al. Assessment of disease severity and outcome in patients with  
1001 systemic light-chain amyloidosis by the high-sensitivity troponin T assay. *Blood* **116**,  
1002 2455-61 (2010).
- 1003 72. Palladini, G. et al. The combination of high-sensitivity cardiac troponin T (hs-cTnT)  
1004 at presentation and changes in N-terminal natriuretic peptide type B (NT-proBNP)  
1005 after chemotherapy best predicts survival in AL amyloidosis. *Blood* **116**, 3426-30  
1006 (2010).
- 1007 73. Kourelis, T.V. et al. Coexistent multiple myeloma or increased bone marrow plasma  
1008 cells define equally high-risk populations in patients with immunoglobulin light chain  
1009 amyloidosis. *J Clin Oncol* **31**, 4319-24 (2013).
- 1010 74. Dittrich, T. et al. AL amyloidosis patients with low amyloidogenic free light chain  
1011 levels at first diagnosis have an excellent prognosis. *Blood* **130**, 632-642 (2017).
- 1012 75. Milani, P. et al. Patients with light-chain amyloidosis and low free light-chain burden  
1013 have distinct clinical features and outcome. *Blood* **130**, 625-631 (2017).
- 1014 76. Sidana, S. et al. Clinical presentation and outcomes in light chain amyloidosis  
1015 patients with non-evaluable serum free light chains. *Leukemia* (2017).
- 1016 77. Kumar, S. et al. Revised prognostic staging system for light chain amyloidosis  
1017 incorporating cardiac biomarkers and serum free light chain measurements. *J Clin*  
1018 *Oncol* **30**, 989-95 (2012).
- 1019 78. Palladini, G. et al. Oral melphalan and dexamethasone grants extended survival  
1020 with minimal toxicity in AL amyloidosis: long-term results of a risk-adapted  
1021 approach. *Haematologica* **99**, 743-50 (2014).
- 1022 79. Kastritis, E. et al. Renal outcomes in patients with AL amyloidosis: Prognostic  
1023 factors, renal response and the impact of therapy. *Am J Hematol* **92**, 632-639  
1024 (2017).
- 1025 80. Kastritis, E. et al. Clinical and prognostic significance of serum levels of von  
1026 Willebrand factor and ADAMTS-13 antigens in AL amyloidosis. *Blood* **128**, 405-9  
1027 (2016).
- 1028 81. Kastritis, E. et al. Growth differentiation factor-15 is a new biomarker for survival  
1029 and renal outcomes in light chain amyloidosis. *Blood* **131**, 1568-1575 (2018).
- 1030 82. Palladini, G. et al. New criteria for response to treatment in immunoglobulin light  
1031 chain amyloidosis based on free light chain measurement and cardiac biomarkers:  
1032 impact on survival outcomes. *J Clin Oncol* **30**, 4541-9 (2012).

- 1033 83. Tandon, N. et al. Impact of involved free light chain (FLC) levels in patients  
1034 achieving normal FLC ratio after initial therapy in light chain amyloidosis (AL). *Am J*  
1035 *Hematol* **93**, 17-22 (2018).
- 1036 84. Kastritis, E. et al. Evaluation of minimal residual disease using next-generation flow  
1037 cytometry in patients with AL amyloidosis. *Blood Cancer J* **8**, 46 (2018).
- 1038 85. Palladini, G. et al. Persistence of Minimal Residual Disease By Multiparameter Flow  
1039 Cytometry Can Hinder Recovery of Organ Damage in Patients with AL Amyloidosis  
1040 Otherwise in Complete Response. *Blood* **128**, 3261-3261 (2016).
- 1041 86. Comenzo, R.L. et al. Dose-intensive melphalan with blood stem cell support for the  
1042 treatment of AL amyloidosis: one-year follow-up in five patients. *Blood* **88**, 2801-6  
1043 (1996).
- 1044 87. Skinner, M. et al. High-dose melphalan and autologous stem-cell transplantation in  
1045 patients with AL amyloidosis: an 8-year study. *Ann Intern Med* **140**, 85-93 (2004).
- 1046 88. Tsai, S.B. et al. High-dose melphalan and stem cell transplantation for patients with  
1047 AL amyloidosis: trends in treatment-related mortality over the past 17 years at a  
1048 single referral center. *Blood* **120**, 4445-6 (2012).
- 1049 89. Batalini, F. et al. High-Dose Melphalan and Stem Cell Transplantation in Patients on  
1050 Dialysis Due to Immunoglobulin Light-Chain Amyloidosis and Monoclonal  
1051 Immunoglobulin Deposition Disease. *Biol Blood Marrow Transplant* **24**, 127-132  
1052 (2018).
- 1053 90. Jaccard, A. et al. High-dose melphalan versus melphalan plus dexamethasone for  
1054 AL amyloidosis. *N Engl J Med* **357**, 1083-93 (2007).
- 1055 91. D'Souza, A. et al. Improved Outcomes After Autologous Hematopoietic Cell  
1056 Transplantation for Light Chain Amyloidosis: A Center for International Blood and  
1057 Marrow Transplant Research Study. *J Clin Oncol* **33**, 3741-9 (2015).
- 1058 92. Nguyen, V.P. et al. Modified High-Dose Melphalan and Autologous Stem Cell  
1059 Transplantation for Immunoglobulin Light Chain Amyloidosis. *Biol Blood Marrow*  
1060 *Transplant* (2018).
- 1061 93. Comenzo, R.L. & Gertz, M.A. Autologous stem cell transplantation for primary  
1062 systemic amyloidosis. *Blood* **99**, 4276-4282 (2002).
- 1063 94. Tandon, N. et al. Revisiting conditioning dose in newly diagnosed light chain  
1064 amyloidosis undergoing frontline autologous stem cell transplant: impact on  
1065 response and survival. *Bone Marrow Transplant* **52**, 1126-1132 (2017).

- 1066 95. Cibeira, M.T. et al. Outcome of AL amyloidosis after high-dose melphalan and  
1067 autologous stem cell transplantation: long-term results in a series of 421 patients.  
1068 *Blood* **118**, 4346-52 (2011).
- 1069 96. Browning, S. et al. Hematologic relapse in AL amyloidosis after high-dose  
1070 melphalan and stem cell transplantation. *Blood* **130**, 1383-1386 (2017).
- 1071 97. Gertz, M.A. et al. Refinement in patient selection to reduce treatment-related  
1072 mortality from autologous stem cell transplantation in amyloidosis. *Bone Marrow*  
1073 *Transplant* **48**, 557-61 (2013).
- 1074 98. Dember, L.M. et al. Effect of dose-intensive intravenous melphalan and autologous  
1075 blood stem-cell transplantation on AL amyloidosis-associated renal disease. *Ann*  
1076 *Intern Med.* **134**, 746-753 (2001).
- 1077 99. Seldin, D.C. et al. Improvement in quality of life of patients with AL amyloidosis  
1078 treated with high-dose melphalan and autologous stem cell transplantation. *Blood*  
1079 **104**, 1888-93 (2004).
- 1080 100. Girnius, S. et al. Hepatic response after high-dose melphalan and stem cell  
1081 transplantation in patients with AL amyloidosis associated liver disease.  
1082 *Haematologica* **94**, 1029-32 (2009).
- 1083 101. Meier-Ewert, H.K. et al. Regression of cardiac wall thickness following  
1084 chemotherapy and stem cell transplantation for light chain (AL) amyloidosis.  
1085 *Amyloid* **18 Suppl 1**, 130-1 (2011).
- 1086 102. Salinaro, F. et al. Longitudinal systolic strain, cardiac function improvement, and  
1087 survival following treatment of light-chain (AL) cardiac amyloidosis. *Eur Heart J*  
1088 *Cardiovasc Imaging* **18**, 1057-1064 (2017).
- 1089 103. Sanchorawala, V. et al. Induction Therapy with Bortezomib Followed by  
1090 Bortezomib-High Dose Melphalan and Stem Cell Transplantation for Light Chain  
1091 Amyloidosis: Results of a Prospective Clinical Trial. *Biol Blood Marrow Transplant*  
1092 **21**, 1445-51 (2015).
- 1093 104. Sanchorawala, V. et al. High-dose intravenous melphalan and autologous stem cell  
1094 transplantation as initial therapy or following two cycles of oral chemotherapy for the  
1095 treatment of AL amyloidosis: results of a prospective randomized trial. *Bone Marrow*  
1096 *Transplant* **33**, 381-8 (2004).

- 1097 105. Sanchorawala, V., Quillen, K., Sloan, J.M., Andrea, N.T. & Seldin, D.C. Bortezomib  
1098 and high-dose melphalan conditioning for stem cell transplantation for AL  
1099 amyloidosis: a pilot study. *Haematologica* **96**, 1890-2 (2011).
- 1100 106. Landau, H. et al. Bortezomib and dexamethasone consolidation following risk-  
1101 adapted melphalan and stem cell transplantation for patients with newly diagnosed  
1102 light-chain amyloidosis. *Leukemia* **27**, 823-8 (2013).
- 1103 107. Dittus, C., Uwumugambi, N., Sun, F., Sloan, J.M. & Sanchorawala, V. The Effect of  
1104 Bone Marrow Plasma Cell Burden on Survival in Patients with Light Chain  
1105 Amyloidosis Undergoing High-Dose Melphalan and Autologous Stem Cell  
1106 Transplantation. *Biol Blood Marrow Transplant* **22**, 1729-1732 (2016).
- 1107 108. Landau, H. et al. Long-term event-free and overall survival after risk-adapted  
1108 melphalan and SCT for systemic light chain amyloidosis. *Leukemia* **31**, 136-142  
1109 (2017).
- 1110 109. Palladini, G. et al. A European collaborative study of cyclophosphamide,  
1111 bortezomib, and dexamethasone in upfront treatment of systemic AL amyloidosis.  
1112 *Blood* **126**, 612-5 (2015).
- 1113 110. Palladini, G. et al. Association of melphalan and high-dose dexamethasone is  
1114 effective and well tolerated in patients with AL (primary) amyloidosis who are  
1115 ineligible for stem cell transplantation. *Blood* **103**, 2936-8 (2004).
- 1116 111. Palladini, G. et al. Melphalan and dexamethasone with or without bortezomib in  
1117 newly diagnosed AL amyloidosis: a matched case-control study on 174 patients.  
1118 *Leukemia* **28**, 2311-6 (2014).
- 1119 112. Venner, C.P. et al. A matched comparison of cyclophosphamide, bortezomib and  
1120 dexamethasone (CVD) versus risk-adapted cyclophosphamide, thalidomide and  
1121 dexamethasone (CTD) in AL amyloidosis. *Leukemia* **28**, 2304-10 (2014).
- 1122 113. Kastiris, E. et al. A Randomized Phase III Trial of Melphalan and Dexamethasone  
1123 (MDex) Versus Bortezomib, Melphalan and Dexamethasone (BMDex) for Untreated  
1124 Patients with AL Amyloidosis. *Blood* **128** (22):646 (2016).
- 1125 114. Bochtler, T. et al. Gain of chromosome 1q21 is an independent adverse prognostic  
1126 factor in light chain amyloidosis patients treated with melphalan/dexamethasone.  
1127 *Amyloid* **21**, 9-17 (2014).

- 1128 115. Bochtler, T. et al. Translocation t(11;14) is associated with adverse outcome in  
1129 patients with newly diagnosed AL amyloidosis when treated with bortezomib-based  
1130 regimens. *J Clin Oncol* **33**, 1371-8 (2015).
- 1131 116. Bochtler, T. et al. Prognostic impact of cytogenetic aberrations in AL amyloidosis  
1132 patients after high-dose melphalan: a long-term follow-up study. *Blood* **128**, 594-  
1133 602 (2016).
- 1134 117. Muchtar, E. et al. Interphase fluorescence in situ hybridization in untreated AL  
1135 amyloidosis has an independent prognostic impact by abnormality type and  
1136 treatment category. *Leukemia* **31**, 1562-1569 (2017).
- 1137 118. Kastritis, E. et al. Addition of cyclophosphamide and higher doses of  
1138 dexamethasone do not improve outcomes of patients with AL amyloidosis treated  
1139 with bortezomib. *Blood Cancer J* **7**, e570 (2017).
- 1140 119. Wechalekar, A. et al. Safety and efficacy of risk-adapted cyclophosphamide,  
1141 thalidomide, and dexamethasone in systemic AL amyloidosis. *Blood* **109**, 457-64  
1142 (2007).
- 1143 120. Dispenzieri, A. et al. Poor tolerance to high doses of thalidomide in patients with  
1144 primary systemic amyloidosis. *Amyloid* **10**, 257-61 (2003).
- 1145 121. Palladini, G. et al. The combination of thalidomide and intermediate-dose  
1146 dexamethasone is an effective but toxic treatment for patients with primary  
1147 amyloidosis (AL). *Blood* **105**, 2949-51 (2005).
- 1148 122. Moreau, P. et al. Lenalidomide in combination with melphalan and dexamethasone  
1149 in patients with newly diagnosed AL amyloidosis: a multicenter phase 1/2 dose-  
1150 escalation study. *Blood* **116**, 4777-82 (2010).
- 1151 123. Santhorawala, V. et al. Melphalan, lenalidomide and dexamethasone for the  
1152 treatment of immunoglobulin light chain amyloidosis: results of a phase II trial.  
1153 *Haematologica* **98**, 789-92 (2013).
- 1154 124. Hegenbart, U. et al. Lenalidomide/melphalan/dexamethasone in newly diagnosed  
1155 patients with immunoglobulin light chain amyloidosis: results of a prospective phase  
1156 2 study with long-term follow up. *Haematologica* **102**, 1424-1431 (2017).
- 1157 125. Kumar, S.K. et al. Lenalidomide, cyclophosphamide, and dexamethasone (CRd) for  
1158 light-chain amyloidosis: long-term results from a phase 2 trial. *Blood* **119**, 4860-7  
1159 (2012).

- 1160 126. Kastritis, E. et al. A phase 1/2 study of lenalidomide with low-dose oral  
1161 cyclophosphamide and low-dose dexamethasone (RdC) in AL amyloidosis. *Blood*  
1162 **119**, 5384-90 (2012).
- 1163 127. Cibeira, M.T. et al. A phase II trial of lenalidomide, dexamethasone and  
1164 cyclophosphamide for newly diagnosed patients with systemic immunoglobulin light  
1165 chain amyloidosis. *Br J Haematol* (2015).
- 1166 128. Palladini, G., Milani, P. & Merlini, G. Novel strategies for the diagnosis and  
1167 treatment of cardiac amyloidosis. *Expert review of cardiovascular therapy* **13**, 1195-  
1168 211 (2015).
- 1169 129. Manwani, R. et al. Rapid hematological responses improve outcomes in patients  
1170 with very advanced (Stage IIIb) cardiac immunoglobulin light chain amyloidosis.  
1171 *Haematologica* (2018).
- 1172 130. Palladini, G. & Merlini, G. What is new in diagnosis and management of light chain  
1173 amyloidosis? *Blood* **128**, 159-68 (2016).
- 1174 131. Warsame, R. et al. Outcomes and treatments of patients with immunoglobulin light  
1175 chain amyloidosis who progress or relapse postautologous stem cell transplant. *Eur*  
1176 *J Haematol* **92**, 485-90 (2014).
- 1177 132. Palladini, G. et al. Presentation and outcome with second-line treatment in AL  
1178 amyloidosis previously sensitive to nontransplant therapies. *Blood* **131**, 525-532  
1179 (2018).
- 1180 133. Milani, P., Gertz, M.A., Merlini, G. & Dispenzieri, A. Attitudes about when and how  
1181 to treat patients with AL amyloidosis: an international survey. *Amyloid*, 1-4 (2017).
- 1182 134. Tandon, N. et al. Treatment patterns and outcome following initial relapse or  
1183 refractory disease in patients with systemic light chain amyloidosis. *Am J Hematol*  
1184 **92**, 549-554 (2017).
- 1185 135. Dispenzieri, A. et al. The activity of lenalidomide with or without dexamethasone in  
1186 patients with primary systemic amyloidosis. *Blood* **109**, 465-70 (2007).
- 1187 136. Santhorawala, V. et al. Lenalidomide and dexamethasone in the treatment of AL  
1188 amyloidosis: results of a phase 2 trial. *Blood* **109**, 492-6 (2007).
- 1189 137. Kastritis, E. et al. A phase 1/2 study of lenalidomide with low-dose oral  
1190 cyclophosphamide and low-dose dexamethasone (RdC) in AL amyloidosis. *Blood*  
1191 **119**, 5384-90 (2012).

1192 138. Kumar, S.K. et al. Lenalidomide, cyclophosphamide, and dexamethasone (CRd) for  
1193 light-chain amyloidosis: long-term results from a phase 2 trial. *Blood* **119**, 4860-7  
1194 (2012).

1195 139. Mahmood, S. et al. Lenalidomide and dexamethasone for systemic AL amyloidosis  
1196 following prior treatment with thalidomide or bortezomib regimens. *Br J Haematol*  
1197 **166**, 842-8 (2014).

1198 140. Palladini, G. et al. Salvage therapy with lenalidomide and dexamethasone in  
1199 patients with advanced AL amyloidosis refractory to melphalan, bortezomib, and  
1200 thalidomide. *Ann Hematol* **91**, 89-92 (2012).

1201 141. Specter, R. et al. Kidney dysfunction during lenalidomide treatment for AL  
1202 amyloidosis. *Nephrol Dial Transplant* **26**, 881-6 (2011).

1203 142. Dispenzieri, A. et al. Activity of pomalidomide in patients with immunoglobulin light-  
1204 chain amyloidosis. *Blood* **119**, 5397-404 (2012).

1205 143. Santhorawala, V. et al. Pomalidomide and dexamethasone in the treatment of AL  
1206 amyloidosis: results of a phase 1 and 2 trial. *Blood* **128**, 1059-62 (2016).

1207 144. Palladini, G. et al. A phase 2 trial of pomalidomide and dexamethasone rescue  
1208 treatment in patients with AL amyloidosis. *Blood* **129**, 2120-2123 (2017).

1209 145. Santhorawala, V. et al. A phase 1/2 study of the oral proteasome inhibitor ixazomib  
1210 in relapsed or refractory AL amyloidosis. *Blood* **130**, 597-605 (2017).

1211 146. Roussel, M. et al. A prospective phase II of daratumumab in previously-treated  
1212 systemic light-chain (AL) amyloidosis. *Blood* **130**, 508 [abstract] (2017).

1213 147. Santhorawala, V. et al. Safety and tolerability of daratumumab in patients with  
1214 relapsed light chain (AL) amyloidosis: preliminary results of a phase II study. *Blood*  
1215 **130**, 507 (2017).

1216 148. Kaufman, G.P. et al. Daratumumab yields rapid and deep hematologic responses in  
1217 patients with heavily pretreated AL amyloidosis. *Blood* **130**, 900-902 (2017).

1218 149. Gertz, M.A. et al. First-in-Human Phase I/II Study of NEOD001 in Patients With  
1219 Light Chain Amyloidosis and Persistent Organ Dysfunction. *J Clin Oncol* **34**, 1097-  
1220 103 (2016).

1221 150. Edwards, C.V. et al. Interim analysis of the phase 1a/b study of chimeric fibril-  
1222 reactive monoclonal antibody 11-1F4 in patients with AL amyloidosis. *Amyloid* **24**,  
1223 58-59 (2017).

- 1224 151. Solomon, A., Weiss, D.T. & Wall, J.S. Immunotherapy in systemic primary (AL)  
1225 amyloidosis using amyloid-reactive monoclonal antibodies. *Cancer Biother.*  
1226 *Radiopharm.* **18**, 853-860 (2003).
- 1227 152. Edwards, C.V. et al. Final Analysis of the Phase 1a/b Study of Chimeric Fibril-  
1228 Reactive Monoclonal Antibody 11-1F4 in Patients with Relapsed or Refractory AL  
1229 Amyloidosis (Abstract 509). *Blood (ASH 59th Annual Meeting and Exposition,*  
1230 *Atlanta, GA, December 9-12)* **130** (2017).
- 1231 153. Richards, D.B. et al. Repeat doses of antibody to serum amyloid P component clear  
1232 amyloid deposits in patients with systemic amyloidosis. *Sci Transl Med* **10** (2018).
- 1233 154. Richards, D.B. et al. Therapeutic Clearance of Amyloid by Antibodies to Serum  
1234 Amyloid P Component. *N Engl J Med* **373**, 1106-14 (2015).
- 1235 155. Tan, N.Y. et al. Catheter Ablation for Atrial Arrhythmias in Patients With Cardiac  
1236 Amyloidosis. *J Cardiovasc Electrophysiol* **27**, 1167-1173 (2016).
- 1237 156. Muchtar, E. et al. Digoxin use in systemic light-chain (AL) amyloidosis: contra-  
1238 indicated or cautious use? *Amyloid*, 1-7 (2018).
- 1239 157. Palladini, G. et al. Holter monitoring in AL amyloidosis: prognostic implications.  
1240 *Pacing Clin Electrophysiol* **24**, 1228-33 (2001).
- 1241 158. Itoh, M. et al. Implantable cardioverter defibrillator therapy in a patient with cardiac  
1242 amyloidosis. *Am J Hematol* **81**, 560-1 (2006).
- 1243 159. Lin, G., Dispenzieri, A., Kyle, R., Grogan, M. & Brady, P.A. Implantable cardioverter  
1244 defibrillators in patients with cardiac amyloidosis. *J Cardiovasc Electrophysiol* **24**,  
1245 793-8 (2013).
- 1246 160. Wright, B.L., Grace, A.A. & Goodman, H.J. Implantation of a cardioverter-  
1247 defibrillator in a patient with cardiac amyloidosis. *Nat Clin Pract Cardiovasc Med* **3**,  
1248 110-4; quiz 115 (2006).
- 1249 161. Rezk, T. et al. Role of implantable intracardiac defibrillators in patients with cardiac  
1250 immunoglobulin light chain amyloidosis. *Br J Haematol* **182**, 145-148 (2018).
- 1251 162. Sayed, R.H. et al. A study of implanted cardiac rhythm recorders in advanced  
1252 cardiac AL amyloidosis. *Eur Heart J* **36**, 1098-105 (2015).
- 1253 163. Ward, J.E. et al. Doxycycline reduces fibril formation in a transgenic mouse model  
1254 of AL amyloidosis. *Blood* **118**, 6610-7 (2011).
- 1255 164. Diomedea, L. et al. A *Caenorhabditis elegans*-based assay recognizes  
1256 immunoglobulin light chains causing heart amyloidosis. *Blood* **123**, 3543-52 (2014).

- 1257 165. Wechalekar, A.D. & Whelan, C. Encouraging impact of doxycycline on early  
1258 mortality in cardiac light chain (AL) amyloidosis. *Blood Cancer J* **7**, e546 (2017).
- 1259 166. Dispenzieri, A., Gertz, M.A. & Buadi, F. What do I need to know about  
1260 immunoglobulin light chain (AL) amyloidosis? *Blood reviews* **26**, 137-54 (2012).
- 1261 167. Kristen, A.V. et al. Improved outcomes after heart transplantation for cardiac  
1262 amyloidosis in the modern era. *J Heart Lung Transplant* **37**, 611-618 (2018).
- 1263 168. Gray Gilstrap, L. et al. Predictors of Survival to Orthotopic Heart Transplant in  
1264 Patients with Light Chain Amyloidosis. *The Journal of Heart and Lung*  
1265 *Transplantation* **33**, 149-56 (2014).
- 1266 169. Davis, M.K. et al. Outcomes after heart transplantation for amyloid cardiomyopathy  
1267 in the modern era. *Am J Transplant* **15**, 650-8 (2015).
- 1268 170. Grogan, M. et al. Long term outcomes of cardiac transplant for immunoglobulin light  
1269 chain amyloidosis: The Mayo Clinic experience. *World J Transplant* **6**, 380-8 (2016).
- 1270 171. Gertz, M.A. et al. Clinical outcome of immunoglobulin light chain amyloidosis  
1271 affecting the kidney. *Nephrol Dial Transplant* **24**, 3132-7 (2009).
- 1272 172. Sattianayagam, P.T. et al. Solid organ transplantation in AL amyloidosis. *Am J*  
1273 *Transplant* **10**, 2124-31 (2010).
- 1274 173. Herrmann, S.M. et al. Long-term outcomes of patients with light chain amyloidosis  
1275 (AL) after renal transplantation with or without stem cell transplantation. *Nephrol*  
1276 *Dial Transplant* **26**, 2032-6 (2011).
- 1277 174. White, M.K., McCausland, K.L., Sanchorawala, V., Guthrie, S.D. & Bayliss, M.S.  
1278 Psychometric validation of the SF-36 Health Survey in light chain amyloidosis:  
1279 results from community-based and clinic-based samples. *Patient Relat Outcome*  
1280 *Meas* **8**, 157-167 (2017).
- 1281 175. Warsame, R. et al. Hematology patient reported symptom screen to assess quality  
1282 of life for AL amyloidosis. *Am J Hematol* **92**, 435-440 (2017).
- 1283 176. Sanchorawala, V. et al. A longitudinal evaluation of health-related quality of life in  
1284 patients with AL amyloidosis: associations with health outcomes over time. *Br J*  
1285 *Haematol* **179**, 461-470 (2017).
- 1286 177. Oliva, L. et al. The amyloidogenic light chain is a stressor that sensitizes plasma  
1287 cells to proteasome inhibitor toxicity. *Blood* **129**, 2132-2142 (2017).

- 1288 178. Song, Y. et al. Blockade of deubiquitylating enzyme Rpn11 triggers apoptosis in  
1289 multiple myeloma cells and overcomes bortezomib resistance. *Oncogene* **36**, 5631-  
1290 5638 (2017).
- 1291 179. Benson, M.D., Liepnieks, J.J. & Kluve-Beckerman, B. Hereditary systemic  
1292 immunoglobulin light-chain amyloidosis. *Blood* **125**, 3281-6 (2015).  
1293  
1294

1295 **Figure 1: Schematic pathways involved in AL amyloid fibril formation.**

1296 A| The usually small and indolent B-cell clone can produce immunoglobulin light chain  $\lambda$  in  
1297 70-80% of cases. Somatic mutations in the light chain variable region *I<sub>GLV</sub>*, cause low  
1298 folding stability and increased protein dynamics, which favors protein misfolding and  
1299 improper aggregation. In addition, interactions between the protein and the tissue  
1300 microenvironment, including extracellular matrix components, shear forces, endoproteases  
1301 and metals, favors protein aggregation and oligomer formation. Cells may promote the  
1302 initial nucleation of the deposits through interaction of the amyloid protein with cell  
1303 membranes. Oligomers and, probably, the misfolded protein exert toxic effects by  
1304 impairing cell function and reducing cell viability in target organs, and can develop to  
1305 highly organized cross- $\beta$  amyloid fibrils. Serum amyloid P component (SAP) protects  
1306 amyloid fibrils from degradation and is ubiquitously present in amyloid deposits.  
1307 Glycosaminoglycans (GAGs) serve as scaffolds and facilitate fibril formation. The  
1308 accumulation of amyloid deposits in parenchymal tissue leads to tissue damage, which  
1309 causes dysfunction of vital organs, moreover amyloid fibrils can cause cell damage and  
1310 catalyze oligomers formation.

1311 B| Light chains derived from certain genes show propensity to target specific organs:  
1312 lambda LV1-44 preferentially targets the heart, lambda LV6-57 the kidney, and kappa  
1313 KV1-33 the liver.

1314

1315 **Figure 2: Kinetics of fibril formation in vitro.**

1316 A specific local concentration of partially folded proteins is necessary for the formation of a  
1317 critical fibrillar nucleus. The critical concentration for nucleation varies, depending on the  
1318 stability of the light chains. During the lag phase, the conditions do not favor protein  
1319 aggregation and fibrils are not formed. However, after the formation of the fibrillar nuclei,  
1320 protein aggregation into cross  $\beta$ -sheet oligomers occurs, leading to fibril formation and  
1321 elongation. The concentration of partially folded proteins necessary for fibril elongation is  
1322 substantially lower than the concentration required for forming the first nuclei. (Courtesy of  
1323 Vittorio Bellotti).

1324

1325 **Figure 3: Organ involvement in systemic AL amyloidosis.**

1326 The symptoms of AL amyloidosis are variable and mimic symptoms observed in common  
1327 conditions of elderly individuals, such as heart failure (fatigue) diabetes mellitus  
1328 (proteinuria and peripheral neuropathy), therefore contributing to late diagnosis. The

1329 presence of heart failure with preserved ejection fraction and thickened ventricular walls  
1330 with low voltages identified using electrocardiography should raise the suspicion of cardiac  
1331 amyloidosis. Kidney involvement is characterized by proteinuria and progressive renal  
1332 failure and manifests as peripheral oedema. The involvement of the gastrointestinal tract  
1333 results in malabsorption and weight loss that can be prominent in some patients, whereas  
1334 involvement of the autonomic nervous system can cause invalidating postural  
1335 hypotension. The presence of prototypic signs such as macroglossia (enlargement of the  
1336 tongue) and periorbital purpura can immediately lead to the right diagnosis. However, such  
1337 signs are uncommon and, more importantly, appear late in the course of the disease,  
1338 frequently when the organ damage caused by amyloid is already irreversible.

1339  
1340

1341 **Figure 4. Diagnostic algorithm for systemic AL amyloidosis**

1342 The presence of heart failure with preserved ejection fraction and/or proteinuria with  
1343 progressive renal failure in a patient with a monoclonal protein should raise the suspicion  
1344 of systemic AL amyloidosis. The involvement of the peripheral and autonomic nervous  
1345 systems as well as hepatomegaly associated with malabsorption and weight loss should  
1346 also trigger the diagnostic process. In patients with a monoclonal protein and abnormal  
1347 free light chain ratio, the unexplained increase in NT-proBNP > 332 ng/L and/or the  
1348 presence of albuminuria >0.5 g/day are diagnostic red flags. In presence of cardiac  
1349 involvement, technetium-labeled bone scintigraphy tracers, such as <sup>99m</sup>Tc-labeled 3,3-  
1350 diphosphono-1,2-propanodicarboxylic acid (DPD) and <sup>99m</sup>Tc-labeled pyrophosphate  
1351 (PYP), help in distinguishing AL amyloidosis from ATTR amyloidosis. In patients without  
1352 serum and urinary monoclonal protein a positive scan (≥ grade 2) is indicative of  
1353 transthyretin amyloidosis. In patients with a monoclonal protein the biopsy of abdominal fat  
1354 and lip salivary glands presents a 85% sensitivity for the diagnosis of AL amyloidosis. In  
1355 patients with a negative biopsy who present with high index of suspicion of heart  
1356 involvement, according to symptoms, echocardiography and ECG, cardiac MRI should be  
1357 used promptly. If positive, cardiac biopsy and possibly amyloid typing is recommended.  
1358 \*Type amyloid with mass spectrometry or immunohistochemistry by very expert amyloid  
1359 pathologist.

1360  
1361  
1362

1363 **Figure 5: Histological evidence of amyloid fibrils in tissue.**

1364 Amyloid deposits are identified in tissue samples using Congo red staining and the  
1365 detection of birefringence using polarized light microscopy. Abdominal fat aspirate from a  
1366 patient with cardiac ATTR amyloid, who had both the TTR variant Val122Ile and an IgG  
1367 kappa MGUS, shows uptake of Congo red (panel A) and birefringence under polarized  
1368 light (panel B) and appears in bright-red in fluorescent light (excitation 497 nm and  
1369 emission 614 nm). Renal tissue from a patient with light chain amyloidosis shows amyloid  
1370 deposits in the glomeruli in bright light (panel D), in polarized light (panel E) and in  
1371 fluorescent light (panel F). Cardiac tissue from a patient with light chain amyloidosis shows  
1372 extensive amyloid deposition with vessel involvement in bright light (panel G), in polarized  
1373 light (panel H) and in fluorescent light (panel I).

1374

1375 **Figure 6: Risk Stratification of patients with AL amyloidosis.**

1376 Survival of 1065 patients diagnosed at the Pavia Amyloidosis Research and Treatment  
1377 Center according to staging systems for survival and progression to dialysis.

1378 A. This cardiac staging system is based on levels of the amino-terminal fragment of  
1379 type B natriuretic peptide (NT-proBNP; with a cutoff level of 332 ng/L) and troponin I (cutoff  
1380 level of 0.1 ng/mL). Patients are classified as stage I, II or III based on the presence of 0,  
1381 1, or 2 markers above the cutoff values, respectively. Troponin T can be used in the  
1382 system instead of troponin I with a cutoff level of 0.06 ng/mL for standard tests and at 54  
1383 ng/L with high-sensitivity assays. Very high levels of NT-proBNP (>8,500 ng/L) identifies  
1384 patients with advanced cardiac dysfunction (Stage IIIb) whereas stage III patients whose  
1385 NT-proBNP is <8,500 ng/L have a better outcome (stage IIIa).

1386 B. The Revised Mayo Clinic Staging system is based on NT-proBNP levels (cutoff  
1387 1,800 ng/L), troponin I levels (cutoff 0.07 ng/mL), and the difference between involved and  
1388 uninvolved circulating free light chain (dFLC; cutoff 180 mg/L). Patients are classified as  
1389 stage I, II, III or IV patients based on the presence of 0, 1, 2, or 3 markers above the  
1390 cutoffs, respectively. Standard troponin T can be used in the system, instead of troponin I,  
1391 with a cutoff at 0.035 ng/mL.

1392 C. The renal staging system is based on proteinuria and estimated glomerular filtration  
1393 rate (eGFR). The percentage/numbers for risk of dialysis for renal stage I, II and III may be  
1394 different according to the treatment modality used. Stage I disease is based on the  
1395 presence of both proteinuria < 5g/24h and eGFR >50 mL/min per 1.73 m<sup>2</sup>; Stage II is

1396 based on either proteinuria >5g/24h or eGFR <50 mL/min per 1.73 m<sup>2</sup>; whereas stage III  
1397 disease is based on both proteinuria >5g/24h and eGFR <50 mL/min per 1.73 m<sup>2</sup>  
1398 In all three staging systems, higher disease stages convey a higher risk of end-stage renal  
1399 disease.

1400

1401 **Figure 7. Risk-adapted treatment of AL amyloidosis.**

1402 Patients at low-risk (representing 20-25% of patients with AL amyloidosis) should receive  
1403 full dose melphalan followed by autologous peripheral blood stem cell transplantation  
1404 (HDM/SCT). Induction therapy with cyclophosphamide, bortezomib and dexamethasone  
1405 (CyBorD) might be used in patients with bone marrow plasma cells (BMPC) >10%, and in  
1406 patients who refuse upfront HDM/SCT. If less than a complete response (CR) is achieved  
1407 3 months after HDM/SCT, consolidation therapy with bortezomib and dexamethasone  
1408 (BDex) should be considered. Patients at intermediate risk (representing ~60% of patients  
1409 with AL amyloidosis) are those who are ineligible for HDM/SCT and without severe cardiac  
1410 involvement. The combination of bortezomib, melphalan and dexamethasone (BMDex)  
1411 can be used to treat patients with the common chromosomal translocation t(11;14), which  
1412 confers a poor response to bortezomib, but these patients are sensitive to standard dose  
1413 melphalan, and can be used in patients with 1q21, whom are poorly responsive to  
1414 standard dose melphalan but who are sensitive to bortezomib. However, melphalan can  
1415 impair stem cell collection in patients who are potential candidates for ASCT - CyBorD  
1416 should be preferred in these patients. Patients presenting with peripheral neuropathy or  
1417 fibrotic lung disease should avoid bortezomib due to its potential neurotoxicity and lung  
1418 toxicity. Patients with severe cardiac involvement (20-25% of patients with AL  
1419 amyloidosis), with very high serum concentration of NT-proBNP (>8500 ng/L) and NYHA  
1420 class ≥ III are considered high-risk, represent an unmet need. Bortezomib-based  
1421 regimens, either attenuated or full dose under close observation in a critical care unit, can  
1422 benefit 30-40% of these patients, although the overall survival is poor (4-7 months). In  
1423 these patients, cardiac transplantation should be considered, followed by ASCT. The  
1424 treatment of relapsing/refractory patients is mostly based on immune-modulatory drugs  
1425 (IMiDs), with pomalidomide emerging as well tolerated and fast acting. Daratumumab is  
1426 highly effective, and based on the outcome of ongoing phase III trial, might be moved to  
1427 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 $\alpha$	Systemic	Hereditary	Kidney, primarily, with obliterative glomerular involvement
A $\beta$ 2M	$\beta$ 2-Microglobulin, wild type	Systemic	Acquired (hemodialysis-related)	Musculoskeletal system
	$\beta$ 2-Microglobulin	Systemic	Hereditary	Autonomous nervous system

\* One family with mutation in the constant region of  $\kappa$  light-chain, with cysteine replacing serine at amino acid residue 131 has been reported<sup>179</sup>

§ 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 <sup>74, 75, 82</sup>	dFLC >50 mg/L	<ul style="list-style-type: none"> <li>• Complete response: negative serum and urine immunofixation and normal FLC ratio</li> <li>• Very good partial response: dFLC &lt;40 mg/L</li> <li>• Partial response: dFLC decrease &gt;50% compared to baseline</li> </ul>
	dFLC 20–50 mg/L	<ul style="list-style-type: none"> <li>• low-dFLC response: dFLC &lt;10 mg/L</li> </ul>
Cardiac <sup>82</sup>	NT-proBNP > 650 ng/L	<ul style="list-style-type: none"> <li>• NT-proBNP decrease &gt;30% and &gt;300 ng/L compared to baseline</li> </ul>
Renal <sup>60</sup>	proteinuria >0.5 g/24h, predominantly albumin	<ul style="list-style-type: none"> <li>• proteinuria decrease &gt;30% compared to baseline (or is &lt; 0.5 g/24h ), in the absence of reduction in eGFR by &gt;25%</li> </ul>

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;