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Proteasome Inhibitors to Treat AL Amyloidosis

James J. Driscoll and Saulius Girnius

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Abstract

Amyloidoses represent a highly heterogeneous group of diseases characterized by the abnormal production and accumulation of abnormal, insoluble amyloid proteins in various tissues leading to organ dysfunction. Light-chain (AL) amyloidosis is the most common form of systemic amyloidosis and is characterized by extracellular deposition of pathologic insoluble fibrillar proteins in organs and tissues. Primary systemic AL amyloidosis (AL) arises from the production of abnormal immunoglobulins (Igs) by clonal plasma cells, such as those associated with the plasma cell dyscrasia multiple myeloma (MM). AL amyloidosis can affect a wide range of organs, most commonly the kidneys, and consequently presents with a range of symptoms. Currently, the most effective treatment is autologous bone marrow transplants with stem cell rescue, but many patients are too weak to tolerate this approach and are ineligible. Novel therapeutic strategies recently used include forms of chemotherapy and targeted therapy similar to those used to treat MM. As a single agent, the proteasome inhibitor bortezomib has notable activity in selected populations of patients with relapsed AL. Here, we discuss recent advances using proteasome inhibitors to improve the outcome of AL amyloidosis patients.

Keywords: Amyloid, light chain, plasma cell, proteasome inhibitor, bortezomib

1. Introduction

Light-chain amyloidosis (AL) is a dyscrasia of clonal origin that results in amyloid fibril deposition within vital organs leading to their progressive dysfunction and ultimately death. The precise molecular events that lead to AL amyloidosis are poorly understood and treatment options based upon the biology of disease that improve patient survival are limited. AL amyloidosis is frequently a challenge to diagnose because of its broad spectrum of symptoms. Clinical manifestations include nephrotic-range proteinuria, hepatomegaly, congestive heart

failure (CHF), and autonomic and sensory neuropathy. Diagnostic advances include development of a serum-free light-chain assay, cardiac magnetic resonance imaging (MRI), and serologic cardiac biomarkers. Treatment advances include the inclusion of the proteasome inhibitor bortezomib. Here, we discuss current and emerging treatment strategies, many focused on proteasome inhibitors, that have evolved or are evolving to prolong survival and preserve organ function in patients with this disease. Finally, we discuss emerging strategies designed to eradicate the clonal cell of origin that may provide further clinical benefit for AL amyloidosis patients.

2. Targeting the ubiquitin + proteasome system in AL amyloidosis

Protein degradation is a highly complex, temporally controlled, highly regulated process that maintains proteostasis in eukaryotic cells [1–5]. In normal and transformed cells, protein degradation pathways fulfill an essential role to maintain many critical pro-survival pathways [1]. Studies by Schoenheimer [1] in the 1930s described the dynamic turnover of individual cellular proteins. Subsequently, it was shown that protein half-lives required energy, in the form of ATP, and that the half-lives of individual proteins varied widely within mammalian cells[2]. Previously, the lysosome had been considered the central site of intracellular proteolysis [3]. Discovery of the small polypeptide protein ubiquitin (Ub) followed as well as experiments demonstrating that Ub is covalently conjugated to target proteins to direct their proteasomal degradation then greatly advanced understanding protein degradation (**Figure 1**) [4, 5]. Ub is covalently linked to protein targets in three sequential steps to target proteins to direct their rapid, ATP-dependent proteasomal degradation. In the first step, Ub is activated by an enzyme referred to as E1. Ub is then transferred from E1 to an E2 Ub-conjugating enzyme, and an isopeptide bond is formed between a lysine residue on the substrate target and the carboxy-terminus glycine of the Ub moiety. E3 Ub protein ligases then recognize Ub-conjugated target proteins [4, 5].

The vast majority of intracellular proteins are degraded by proteasomes in eukaryotic cells. The 26S proteasome is high-molecular-weight, ATP-dependent structure that consists of a 20S catalytic core particle (CP) capped at the ends by 19S regulatory particles (RP) (**Figure 2**) [6–10]. The proteasome serves as the catalytic core of the Ub-proteasome system (UPS) to degrade short-lived and denatured proteins and was the first component of the Ub-proteasome pathway to be targeted therapeutically. Bortezomib is a selective, boron-containing reversible inhibitor of the proteasome that induces apoptosis in a number of different cancer cells. Bortezomib is a potent small molecule that binds reversibly to the proteasome β-5 subunit to inhibit the chymotryptic-like (Ct-L) activity (**Figure 3**). The anti-tumor effect of bortezomib was evident in a multitude of cell lines and xenograft models from different cancer types [11–15], including malignant plasma cells. Bortezomib has demonstrated substantial benefit in monotherapy or in combinations that induce chemo- or radiosensitization [11]. Federal Drug Administration (FDA)-approval of bortezomib (Velcade, Millennium-Takeda Oncology Co., Cambridge, MA) represented a major advance in the treatment of MM [11, 12].

Bortezomib is the first proteasome inhibitor to change the natural history of a hematologic malignancy. However, clinical efficacy in the treatment of solid tumors has not been achieved.
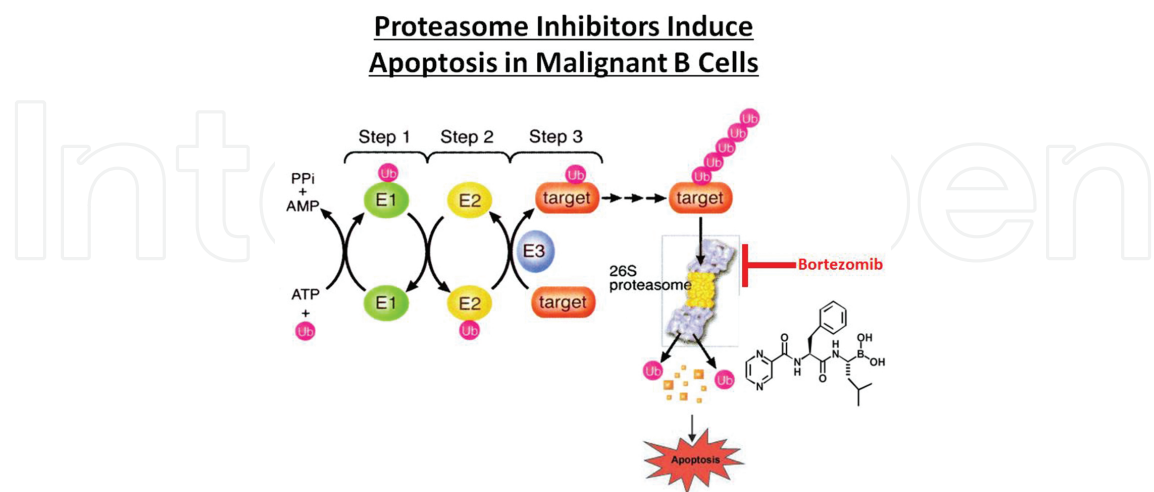

Figure 1. Schematic representation of the Ub + proteasome system. The UPS pathway for protein degradation in eukaryotic cells comprises: (1) a series of enzymes [E1, activation; E2, conjugation; E3, ligation] that covalently modify proteins with a polyubiquitin tag for recognition and targeted degradation and (2) the 26S proteasome, a 2 MDa multicatalytic enzyme complex that hydrolyzes the polyubiquitin-tagged proteins into short polypeptides, typically seven to nine amino acids in length. The proteasome degrades unwanted proteins by recognizing specific polyubiquitin tags covalently attached to these proteins. Inhibition of the proteasome catalytic core by bortezomib leads to the unwanted accumulation of ubiquitinated proteins and culminates in apoptosis.
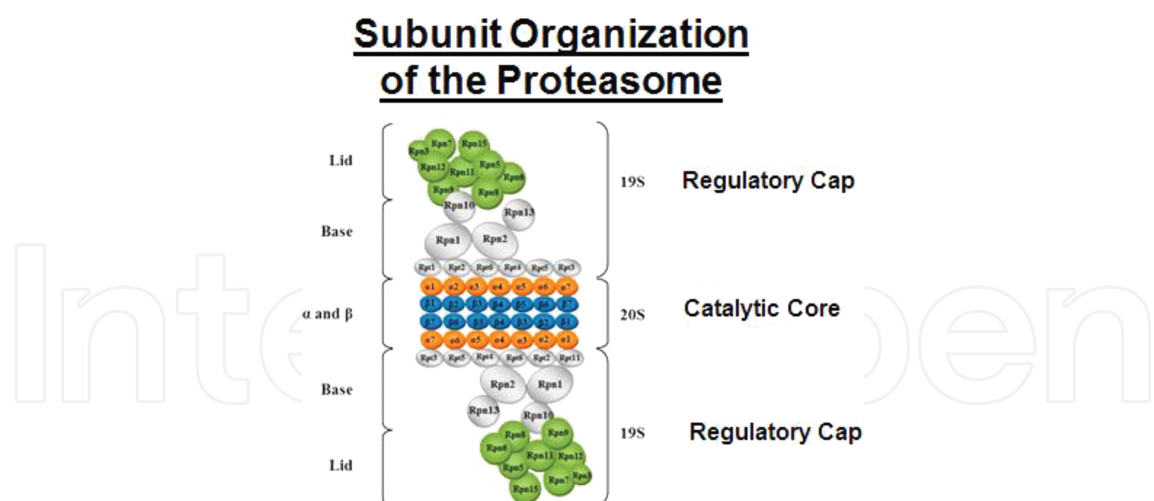

Figure 2. Subunit organization of the 26S proteasome. The 26S proteasome is a eukaryotic ATP-dependent, dumbbell-shaped protease complex with a molecular mass of approximately 2000 kDa. It consists of a central 20S proteasome, functioning as a catalytic machine, and two large V-shaped terminal modules, having possible regulatory roles, composed of multiple subunits of 25–110 kDa attached to the central portion in opposite orientations. Shown are the 20S proteasome catalytic particles (CP) and the 19S regulatory particles (RP). The 19S regulator is bound to either one or both ends of the 20S proteasome and stimulates hydrolytic activity of the 20S proteasome. The 19S RP enables ATP-dependent degradation of ubiquitinated proteins and supports elevated peptidase activity but not ubiquitin-conjugate degradation.
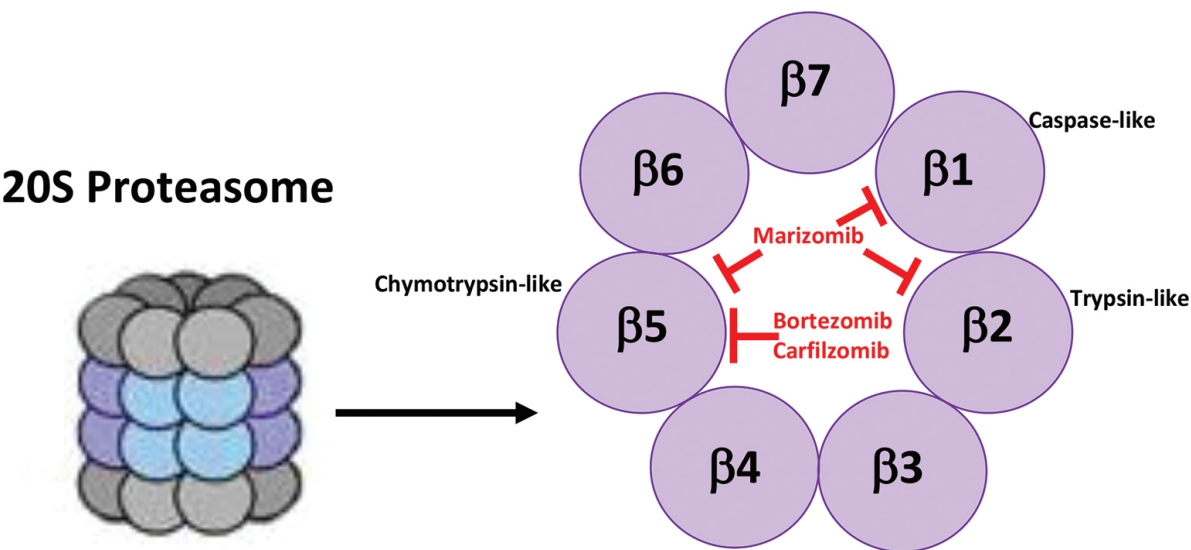
Figure 3. The 20S proteasome CP houses three pairs of catalytically active subunits, β1, β2, and β5, that exhibit protein substrate cleavage preferences referred to as caspase-like (C-L), trypsin-like (T-L), and chymotrypsin-like (CT-L), respectively, and which work in concert to degrade protein substrates. Substrate hydrolysis by the 20S CP commences with recognition of amino acid side chains by sequential binding pockets proximal to the proteolytic active site. Bortezomib forms a reversible, non-covalent adduct at the active site of the β-5 subunit. Carfilzomib has irreversible binding properties for the same active site of the β-5 subunit. Marizomib is a β-lactone-γ-lactam that binds slowly with sustained inhibition of the proteasome β-1, β-2, and β-5 active sites.

The aim of current treatment strategies for AL is to inhibit production of insoluble amyloidogenic immunoglobulin light-chain fragments and ultimately restore organ function [16–18]. AL and MM are both clonal plasma cell dyscrasias and, therefore, AL treatment is typically based on therapies that have shown efficacy in MM [19, 20]. The depth of hematologic response and complete response (CR) has been linked with improved organ function in AL patients and improved overall survival (OS) in MM [21–23]. Intensive therapy with high-dose melphalan and stem cell transplantation has been shown to be highly effective in AL, but a risk-adapted strategy may be needed to reduce mortality and toxicity [24, 25]. In addition, similar to MM, side effects with treatment are evident and drug resistance emerges even in patients that do respond initially.

Second-generation proteasome inhibitors offer a number of potential advantages over bortezomib. The newer proteasome inhibitors may provide improved target specificity, safety, tolerability, and the capacity to overcome bortezomib resistance (**Table 1**). Second-generation proteasome inhibitors include the recently FDA-approved epoxyketone carfilzomib [26], and agents in clinical development that include ONX-0912 [27], Ixazomib (Ninlaro) [28] (Millennium-Takeda), Marizomib [29] (NPI-0052), and CEP-18870 [30] (Cephalon). In contrast to bortezomib, carfilzomib is an irreversible proteasome inhibitor. Ixazomib is an orally bioavailable reversible proteasome inhibitor that is immediately hydrolyzed to its active form, MLN2238 after conversion in aqueous solutions. Ixazomib binds preferentially to the proteasome β-5 active site to inhibit the chymotrypsin-like activity. Ixazomib and CEP-18770 are reversible inhibitors of the proteasome Ct-L activity that exhibit inhibitory activity comparable to bortezomib. The differences improved anti-tumor activity.

Inhibitor	Structural Class	Inhibitor Type inhibited	Activity	Stage of Development	Route of administration
Bortezomib (Millennium)	Peptide- boronic acid	R	Ct-L	FDA-approved Immuno	IV
Carfilzomib (Onyx)	Tetrapeptide epoxyketone	I	Ct-L	FDA-approved	IV
MLN9708 (Millennium	Peptide boronic acid	R	Ct-L	Phase I	IV/po
Marizomib (Nereus)	β-lactone- γ-lactam	I	Ct-L, Tryptic, Caspase-like	Phase Ib	IV
ONX-0912 (Onyx)	Peptide epoxyketone	R	Ct-L	Phase I	IV/po
Cep-18870 (Cephalon)	Peptide- >boronic acid	R	Ct-L	Phase I–III	IV/po

R = reversible, I = irreversible; Ct-L = chymotryptic-like; Immuno = immunoproteasome, IV = intravenous; po = oral

Table 1. Agents to target the proteolytic activities within the proteasome complex.

Carfilzomib is a tetrapeptide epoxyketone that also irreversibly inhibits the Ct-L activity of proteasomes [31–36]. Carfilzomib yields a more sustained inhibition compared to bortezomib and has been shown to promote cell death in bortezomib-resistant cells. ONX-0912 is an orally bioavailable analog of carfilzomib that has been investigated in early phase trials to study the effect on solid tumors. ONX-0912 irreversibly inhibits β-5 activity and in xenograft models was shown to reduce tumor growth and prolong survival. ONX-0914 is an immuno-proteasome-specific inhibitor with potential to treat both cancers and autoimmune diseases. Marizomib (NPI-0052) irreversibly inhibits the β-5 subunit and is a natural β-lactone derived from *Salinospora tropica.* Marizomib inhibits the major catalytic activities (Ct-L, tryptic-like, and caspase-like) of proteasomes which may yield a long-term benefit to preclude resistance (**Figure 3**). Thus, the benefit of second-generation proteasome inhibitors may be due to their ability to act as irreversible inhibitors and to inhibit multiple active sites within the proteasome.

3. Clinical evaluation of proteasome inhibitors for AL amyloidosis treatment

Intensive therapy with high-dose melphalan (a chemotherapy drug belonging to the class of nitrogen mustard alkylating agents) and stem cell transplantation (SCT) have been combined as a regimen (MEL-SCT) and used as an effective therapy in AL amyloidosis. MEL-SCT is used as a risk-adapted approach that is necessary to minimize treatment-related mortality

(TRM). Therefore, up to 82% of persons with AL amyloidosis may be ineligible for MEL-SCT based upon age, performance status, and severity of cardiac involvement. Wechalekar et al. [37] reported preliminary observations with bortezomib and demonstrated its effectiveness in a heavily pre-treated population, with an 80% hematologic overall response (OR) rate, including 15% hematologic CR rates. A subsequent phase I/II study of single agent bortezomib in relapsed/refractory aL amyloidosis showed excellent tolerance to both once- and twice-weekly [38]. Importantly, the median time to response in the twice-weekly arm was only 0.7 months. One-year hematologic response rates were 72.2 and 74.6%, one-year response rates were 93.8 and 84.0%, and one-third of patients exhibited a CR. The median OS was 61.1 months [39]. Rates of neuropathy were as high as 35% in the twice-weekly, with 9% rate of discontinuation and 6% rate of dose reductions.

Deregulating the ubiquitin-proteasome pathway may affect the heart, causing plaque instability, altered intracellular signal transduction resulting in decreased myocardial cytoprotection, desensitization of adrenergic receptors, and accumulation of unfolded proteins impairing cardiac function [40]. Although cardiac toxicity was described in small series, case reports, and clinical trials, subsequent meta-analysis did not support an increase in cardiotoxicity in multiple myeloma (MM) [41]. Early sudden death has been described in amyloid cardiomyopathy in persons treated with bortezomib. The Greek and UK groups demonstrated increased rates of early cardiac death in those treated with bortezomib, when compared to lenalidomide [42, 43]. However, sudden death is not uncommon in advanced amyloid cardiomyopathy. Since bortezomib typically has a shorter time to hematologic response, it is used more frequently than lenalidomide in advanced cardiac amyloidosis. Therefore, increased rates of sudden death may be related to patient selection, rather than a class effect from proteasome inhibitors.

Since bortezomib demonstrated such great promise, several studies explored the clinical efficacy in triplets, including cyclophosphamide, bortezomib, and dexamethasone (CyBorD). These retrospective studies reported on 17 and 43 patients, with hematologic response rates of 81–94% and hematologic CR rates of 39.5–71.5% [44, 45]. At least half of the patients had symptomatic cardiac involvement and many had a cardiac-specific response. However, a retrospective study from the UK demonstrated poorer outcomes than the two aforementioned studies [46]. Of 230 patients, a hematologic response rate was 60%, which decreased to 42% in those with advanced cardiac stage III patients. Cardiac response was achieved in only 17% of patients. Unfortunately, CyBorD is associated with grade 3 and grade 4 toxicities in 50% of patients.

Bortezomib has been combined with melphalan and dexamethasone, possibly with more promising outcomes than CyBorD (**Table 2**). A prospective, multi-center phase II trial of bortezomib, melphalan, and dexamethasone (VMD) in newly diagnosed or relapsed AL amyloidosis showed hematologic response rates of 94%, with CR of 38%. However, this was a small trial and 52% of patients required dose reduction despite an excellent performance status, questioning whether such an impressive response can be replicated off trial [47]. A subsequent phase I trial demonstrated improved safety and greater tolerability [48]. Using prospectively collected data, the Pavia group demonstrated higher response rates with VMD,

when compared to CyBorD (p=0.033) or melphalan/dexamethasone alone ($p = 0.010$) [49]. While the exposure to upfront bortezomib is associated with longer OS, a difference between CyBorD and VMD could not be detected. A bortezomib/dexamethasone backbone is necessary in persons ineligible for melphalan-stem cell transplantation (MEL-SCT), and it is not clear whether melphalan or cyclophosphamide should be added. The addition of melphalan is supported with more prospective data, but higher cumulative doses are associated with leukemia, and melphalan should be avoided in persons who are borderline candidates for autologous stem cell transplantation.

Regimen	Type of Study	Patients	Population Size	ORR (%)	CR (%)	Dose Adjustment/ discontinuation (%)
BD[36]	Retrospective	R/R	18	94	44	61
BD[27]	Retrospective	R/R	20	80	15	40
BD[43]	Retrospective	ND	18	81	47	
BD[43]	Retrospective	R/R	76	68	20	
BD[28]	Phase I/II	R/R	70	69	37.5	53
CyBorD[34]	Retrospective	ND	17	94	71	
CyBorD[35]	Retrospective	ND, R/R	43	81	42	
CyBorD[36]	Retrospective	ND	230	60	23	
VMD[37]	Phase I/II	ND, R/R	30	94	38	52
VMD[38]	Phase I/II		9			
ID[40]	Phase I	R/R	22	53	11	
KD[41]	Phase I	R/R	12	77	0	

AL = light chain; ORR = overall response rate; CR = hematologic complete response; BD = bortezomib/dexamethasone; ND = newly diagnosed; CyBorD = cyclophosphamide/bortezomib/dexamethasone; VMD= bortezomib/melphalan/dexamethasone; ID= ixazomib/dexamethasone; KD= carfilzomib/dexamethasone

Table 2. Proteasome inhibitors used in treatment regimens for AL amyloidosis.

The use of ixazomib in a phase I study in relapsed/refractory AL amyloidosis was reported only in abstract form [50]. Of the 22 patients enrolled, 11 patients were previously treated with bortezomib and 15 with cardiac involvement. Seventy-seven percent were able to reach maximum-tolerated dose. A hematologic response was obtained in 53%, including 11% CR. An organ-specific cardiac response was seen in three of nine patients. The 2-year OS was 57%, up to 85% in proteasome inhibitor naïve patients. Tourmaline-AL1, a phase III trial for ixazomib/dexamethasone vs. physician's choice is currently ongoing and accruing patients.

Carfilzomib is another second-generation proteasome inhibitor that is being investigated as a potential treatment in AL amyloidosis. A phase I dose escalation study of carfilzomib in AL

amyloidosis in a bortezomib-exposed population enrolled 12 patients [51]. A hematologic OR rate of 77% was reported and no dose-related toxicity was noted in carfilzomib doses as high as 36 mg/m^2. However, three significant cardiac events were noted, including ventricular tachycardia, grade 4 restrictive cardiomyopathy, and a grade 3 drop in the left ventricular ejection fraction. Although promising, second-generation proteasome inhibitors may not provide significant long-term benefit and overcome therapeutic resistance in refractory patients. Numerous mechanisms of resistance have been proposed, for example, mutations of PSMB5, upregulation of other proteasome subunits, alterations of gene and protein expression in stress response pathways, induction of autophagy, and an increase in anti-apoptotic pathways as well as multidrug resistance pathways. Hence, a number of novel therapeutic strategies for AL amyloidosis are under development [52, 53]. Currently, treatment choices remain highly individualized and are dependent on a careful assessment of performance status and organ function.

4. Targeting the clonal cell of origin as a treatment strategy for AL amyloidosis

The incidence of AL amyloidosis is similar to that of Hodgkin's lymphoma and chronic myelogenous leukemia (CML). Approximately 5–12 individuals/million/year are affected, although autopsy studies suggest a higher incidence. Amyloidosis is a monoclonal plasma cell disorder in which the secreted monoclonal Ig protein forms insoluble fibrillar deposits in one or more organs. In nearly all cases, the deposits contain Ig light (L) chains or L-chain fragments. AL is related to both MM and monoclonal gammopathy of undetermined significance (MGUS), a pre-malignant condition that nearly uniformly precedes MM. These monoclonal plasma cell disorders are categorized according to the total body burden of monoclonal plasma cells. When this burden is large, the diagnostic criteria for MM are fulfilled; when this burden is lower, MGUS is diagnosed. The plasma cell burden is typically low at 5–10%, and in ~10–15% of patients, AL amyloidosis occurs in association with MM.

5. Concluding remarks

While targeting proteostasis is a highly effective strategy to treat plasma cell dyscrasias such as AL, more effective agents are needed to improve organ dysfunction and advance patients to SCT. Moreover, similar to MM, high-risk forms of disease exist which do not respond to bortezomib and for those that do respond drug resistance eventually emerges. While the explosion of novel agents with activity in MM holds promise for the care of patients with AL amyloidosis, a commitment specifically to the clinical investigation of AL amyloidosis is needed to improve patient outcomes. Therefore, there is an urgent and unmet need for more effective therapeutic agents based upon the biology of the disease that increase patient survival.

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