



High-Dose Therapy for the Treatment of Primary Systemic Amyloidosis

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AL amyloidosis involving major organs can result in progressive debility, organ failure and early death.⁽¹⁻³⁾ Minimal progress had been made in reliably reversing this pathology until the mid 1990s when AL patients received dose-intensive intravenous melphalan and autologous hematopoietic cell transplantation (HCT).⁽⁴⁻⁷⁾ The effectiveness of HCT in reversing the amyloid deposition syndromes in two-thirds of patients surviving at one year has been documented at numerous centers (**Table 1**), and amyloid scans have demonstrated resorption of AL depositions in association with reduction or elimination of the clonal plasma cell disorder that is the root cause of AL.^(15,18) The performance status and quality of life of patients whose AL regresses after HCT can also

dramatically improve. However, the mortality within 100 days of HCT averages 27% in toto (range 13% to 44% for references 6 thru 17), making HCT unacceptably morbid (**Table 1**). In the following sections, the lessons learned from treating AL with HCT are summarized and guidelines offered for refining patient selection. Three areas are emphasized: outcomes and toxicities; the pathophysiology and genetics of AL; and the use of blood stem cells. Challenges to future progress are discussed in closing.

Outcomes and Toxicities

As a result of treating AL with HCT we have, first and foremost, re-learned the lesson that survival for AL patients is a function of the type and extent of amyloid-

Table 1. Summary of hematopoietic cell transplantation results in primary systemic amyloidosis.

Author (ref)	Patients treated, N	Peri-transplant deaths, N	Patients surviving at one year, N	Amyloid organ responses, N
Case Reports ⁽⁸⁻¹²⁾	6*	0	6	5
<i>Larger Series:</i>				
Comenzo 1998 ⁽⁶⁾	23	3	21	12
Comenzo 1999 ⁽¹³⁾	28	6	15	7
Moreau 1998 ⁽¹⁴⁾	21	9	12	10
Gilmore 1999 ⁽¹⁵⁾	27	8	14	8
Gertz 1999 ⁽¹⁶⁾	19	4	12	9
Amoura 1998 ⁽¹⁷⁾	9	4	5	4
Total	133	34 (26%)	85	55 (65%)

*Includes allogeneic HCT in two patients

related organ involvement.^(19,20) This lesson has numerous corollaries with respect to prognostic factors and patient selection. Second, we have learned that the response of amyloid-related organ involvement is generally linked to hematologic response, itself a function of features of the underlying clonal plasma cell disorder. Third, we have learned that, in this patient population, treatment-related toxicities can escalate, and amyloid-related complications can pose prominent clinical challenges during the peritransplant period.

Organ involvement and post-transplant survival

In the majority of transplant series for AL listed in Table 1, standard criteria for autologous HCT were employed to determine eligibility for treatment (age < 61 years, performance status 0-3, left ventricular ejection fraction (LVEF) > 50%, pulmonary diffusion capacity (DLCO) > 50% predicted, and adequate hepatic and renal function) with the notable exception being the lower dose melphalan trial.⁽¹³⁾ And, despite the use of these stringent screening criteria, the treatment-related mortality of AL patients was several fold higher than the 5% to 10% mortality currently reported for non-Hodgkin's lymphoma. Refinement of patient selection for the transplantation of AL patients, then, has become a priority.⁽²¹⁾

An appreciation of the impact of amyloid-related organ involvement on survival provides an important first step in refining patient selection. In **Figures 1** and **2**, Kaplan-Meier plots for reported trials^(6,13) are updated showing that AL patients with symptomatic cardiac amyloid or with more than two major systems involved have significantly poorer overall survival. The treatment-related deaths in these and other series have been due to cardiac complications, gastrointestinal bleeding, sepsis and, rarely, visceral rupture; moreover, multiorgan fail-

ure (MOF) with intractable hypotension has frequently been observed pre-mortem, likely a variant of the systemic inflammatory response syndrome (SIRS).^(22,23) Deposits of amyloid can compromise visceral organ function and, therefore, limit visceral reserve. The toxicities of dose-intensive therapy are to some degree inversely proportional to visceral reserve. Progressive organ failure, endothelial cell damage, gut mucosal leakiness, and the elaboration of pro-inflammatory cytokines likely play roles in the MOF syndrome often seen in association with early death in AL transplant patients, a clinical picture consistent with SIRS. However, some patients in both of these poor risk groups survived treatment and improved, and useful intra-group distinctions can be made. For example, **Figure 3A** depicts the significantly better overall survival of dominant cardiac amyloid patients with uncomplicated symptomatology. Patients with uncomplicated symptomatology had no history of syncope, arrhythmias or recurrent pleural effusions; patients with complicated symptomatic cardiac amyloid had one or more of these clinical features. Patients with uncomplicated symptomatology can appropriately be considered for HCT clinical trials while the former group should not routinely be evaluated with HCT in mind. Similarly, **Figure 3B** depicts the overall survival of patients with more than two major systems involved as a function of age. Although overall survival was not significantly better in younger patients, the only surviving (and improved) patients in this group were 50 years old or younger; it is not unreasonable to consider those patients candidates for HCT clinical trials designed to be less toxic. In addition, the better risk cardiac and multisystem patients are appropriate candidates for phase III trials that seek to determine whether HCT is more effective than conventional oral chemotherapy (melphalan and prednisone).

The treatment-related mortality of good risk patients who have one or two major systems involved and do not have symptomatic cardiac amyloid (although they may have evidence of asymptomatic cardiac involvement) is typical of autologous HCT. Regimen-related toxicities may be somewhat greater, and they are also still at risk for rare amyloid-related complications such as splenic rupture.^(4,24) Therefore, it is reasonable to consider autologous HCT a standard therapy for these good risk patients, although whenever possible they should be treated on clinical trials designed to evaluate interventions aimed at increasing the complete hematologic response rate. Although age and gender do not significantly influence outcome, it should be noted that there is limited experience with good risk AL patients older than 61 years being treated with 200 mg/m² of melphalan; such patients frequently receive lower doses, as do patients on peritoneal or hemodialysis.

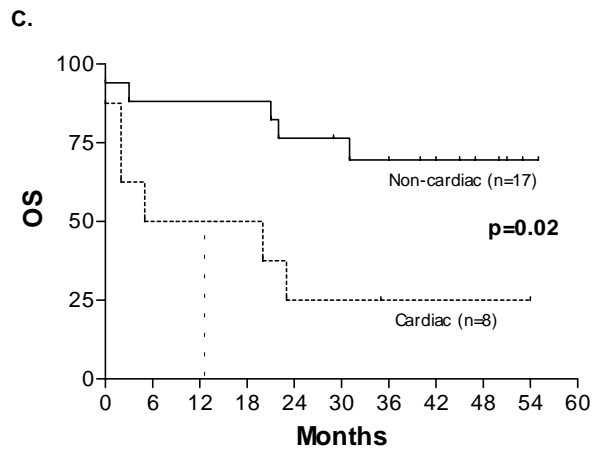
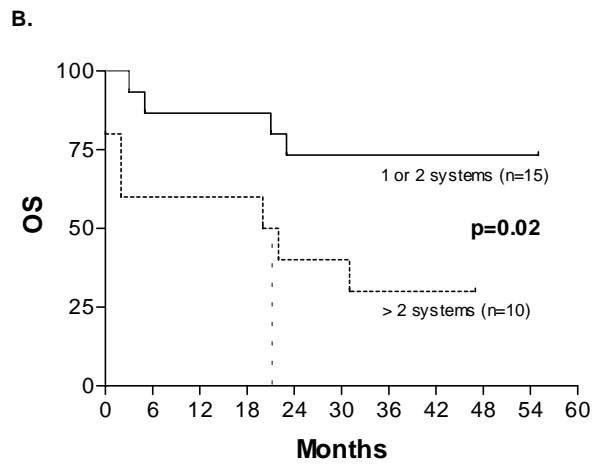
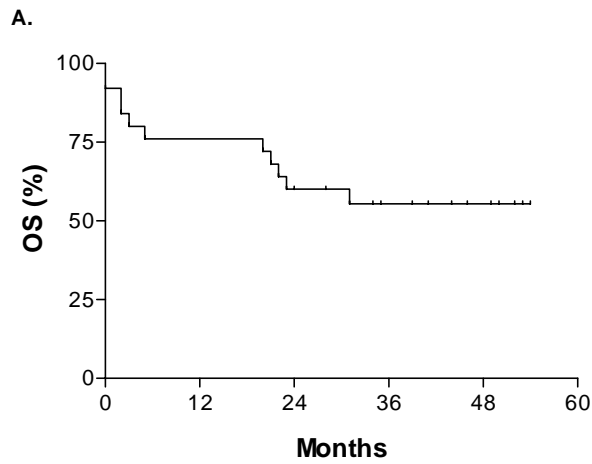


Figure 1. The overall survival (OS) of 25 AL patients treated with hematopoietic stem cell transplantation and melphalan, 200 mg/m²,⁽⁶⁾ is updated with a median follow-up of over 2.5 years. Depicted are OS (A), survival based on number of major organs involved (heart, kidneys, liver and peripheral nerves) (B), and survival based on dominant organ involvement (C).

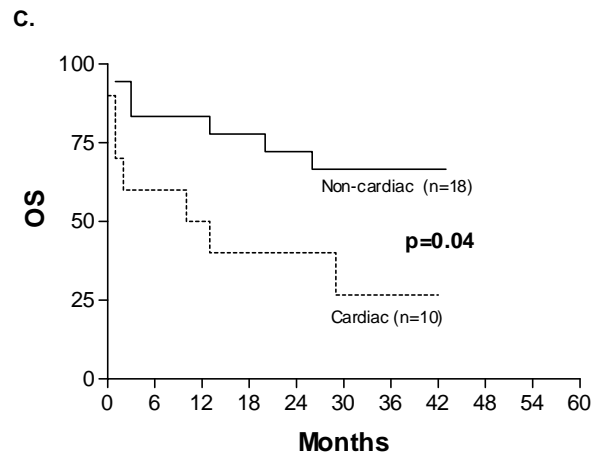
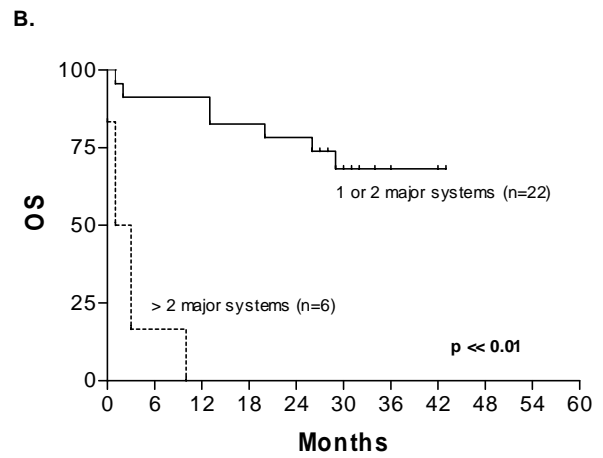
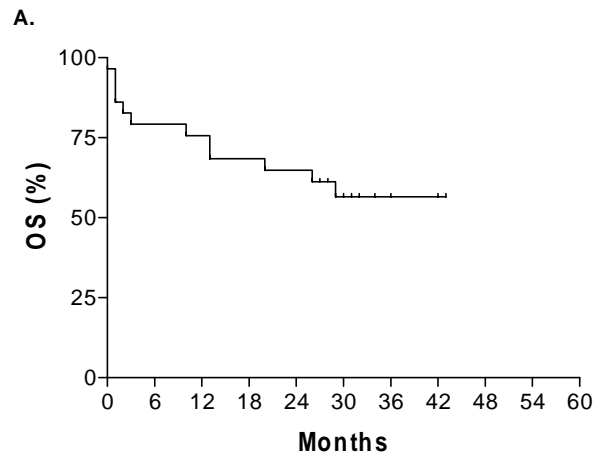


Figure 2. The survival of 28 AL patients treated with hematopoietic stem cell transplantation and melphalan, 100 mg/m²,⁽¹³⁾ is updated with a median follow-up of over 2 years. Categories are as in Figure 1.

Hematologic responses and their significance

The significance of the hematologic response to HCT can best be appreciated by examining the experience at Boston Medical Center from 6/94 through 1/98. The background of that experience has been described in detail.⁽²⁵⁻²⁷⁾ During that period, 113 AL patients were offered HCT; of these, 103 were treated, including the patients described in refs 6 and 13. They were equally divided with respect to melphalan dose between 200 and 100-140 mg/m². Patients who received 100 or 140 mg/m² did so because of age > 61 years, LVEF < 50%, DLCO < 50% predicted, or renal failure requiring dialysis. All patients were mobilized with growth factor alone and supported with blood stem cells or, rarely, blood stem cells and bone marrow. Patients were evaluated at 3 and 12 months post-transplant and annually thereafter for hematologic response to HCT.

A complete hematologic response (CR) required a bone marrow biopsy without evidence of the prior clonal plasma cell disorder based on immunohistochemical staining of plasma cells for κ and λ light chains, and immunofixation studies of serum and urine (concentrated 10x) without evidence of previously identified monoclonal proteins. Patients were categorized as having complete hematologic responses (CR) or persistent clonal disease (PD). Amyloid-related organ involvement was also evaluated at these intervals by objective criteria (6,25). The significance of the CR/PD distinction with respect to overall survival is depicted in **Figure 4**. The links between hematologic response and response of amyloid-related organ involvement are shown in **Figure 5**. Patients achieving a CR had better overall survival than those with PD, particularly among patients receiving 100-140 mg/m² of melphalan.

The CR rate, not surprisingly, was dose-related; however, the probability of achieving a CR was related to the clonal light chain isotype and the presence or absence of a serum M protein (**Table 2**). Patients with κ clones were more likely to achieve CR with HCT than those with λ clones; moreover, among those with λ clones, patients without serum M proteins were more likely to achieve CR than patients with them. The frequency of improvement of amyloid-related organ involvement is linked to the achievement of a CR although patients with PD can also experience improvement (**Figure 5**). Therefore, by combining organ-related and hematologic variables, one can crudely stratify patients (good-fair-poor) with respect to likely outcome with HCT (**Table 3**). Of note, relapses of the clonal plasma cell disease occurred 1, 2 and 4 years post-transplant with an apparent rate of between 25% and 50% at 4 years; interestingly, not all patients whose clonal disease relapsed experienced simultaneous progression of amyloid organ syndromes.

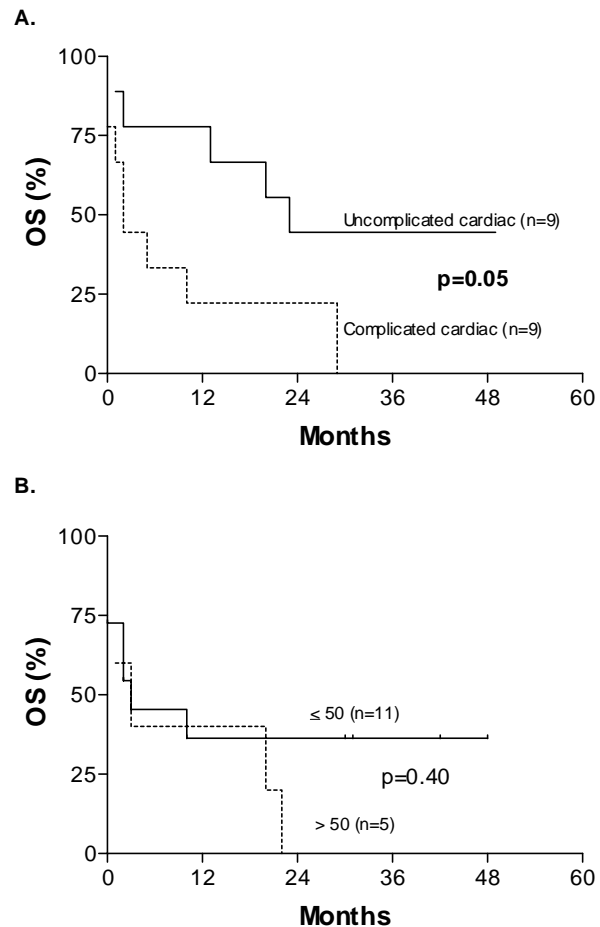


Figure 3. The survival curves of two categories of poor risk AL patients, cardiac patients (A) and patients with > two major organs involved (B) pooled from reported trials^(6,13) are depicted. In 3A, survival is significantly better for patients with dominant cardiac involvement but uncomplicated symptomatology, i.e., without findings of syncope, arrhythmias or recurrent pleural effusions, with a median survival of nearly 2 years. In 3B, only patients younger than 50 (4 of 11) survived in the group with more than two major organs involved.

Toxicities and complications

It was predictable that regimen-related toxicity (RRT) would be more prominent in this patient population, given the impaired visceral reserve, complex coagulopathies and vasculopathy associated with AL. To some degree the frequency and grade of RRT are related to the dose of melphalan as indicated by a comparison of toxicities (SWOG ≥ 2) from cohorts treated with 200 or 100 mg/m² of melphalan (**Table 4**).^(6,13) Of particular note, the degree of gastrointestinal toxicity with 200 mg/m² of melphalan is striking, as are the higher rates of edema and bleeding with that dose.

Involvement of the gastrointestinal tract with AL can be focal or diffuse, and symptoms usually are linked to the location and extent of AL deposits.⁽²⁸⁾ The entire length of the gastrointestinal (GI) tract may be involved.

Macroglossia, which occurs in about 10% of patients, can be massive and produce inability to eat or drink normally, airway obstruction and sleep apnea. Achalasia, hematemesis, gastroparesis, and pseudo-obstruction are among the many possible manifestations of GI amyloid. Extensive amyloid infiltrates of the submucosa of the stomach or lower tract increase the potential for severe mucositis with hemorrhage, while neuropathic compromise of the enteric plexus often results in atony, persistent post-transplant nausea and failure to thrive. Therefore, pre-transplant planning becomes essential; evaluation should include a detailed review of GI signs and symptoms, serial stool guaiacs, endoscopic studies to define pathology when indicated by symptoms or other findings, and a complete assessment of coagulation status. Vague or atypical left-sided abdominal or shoulder pain should raise a concern about occult splenic hemorrhage and lead to consideration of imaging the abdomen. Also, a plan should be developed for the management of GI amyloid, if present, with respect to extended antiemetic regimens, prophylaxis and management of GI bleeding and re-feeding. Proton-pump inhibitors such as omeprazole may be useful. We have found the following regimen useful to reduce the delayed emesis associated with dose-intensive intravenous melphalan. Our delayed-emesis regimen begins the day after stem cell infusion and consists of dexamethasone 2 to 4 mg bid, lorazepam 0.5 to 1.0 mg bid to tid and prochlorperazine 5 mg bid to tid and is maintained for a week. If breakthrough nausea and vomiting occur, daily granisetron can be used in place of prochlorperazine.

Major GI bleeds can present atypically as new onset

Table 2. Hematologic response as a function of clonal plasma cell disease.

Feature	CR (n)	PD (n)	p-value
Clonal isotype			
κ (n=11)	0.91 (10)	0.09 (1)	< 0.01
λ (n=73)	0.42 (31)	0.58 (42)	
Serum M-protein			
Present (n=48)	0.42 (20)	0.58 (28)	0.04
Absent (n=36)	0.61 (22)	0.39 (14)	

Table 3. Combined prognostic factors.

Amyloid Disease	Clonotypic Features		
	Kappa Clone	Negative Serum Immunofixation	Lambda Positive Serum Immunofixation
≤ 2 systems	Good	Good	Good to fair
Dominant cardiac	Fair to poor	Fair to poor	Poor
> 2 systems	Fair to poor	Fair to poor	Poor

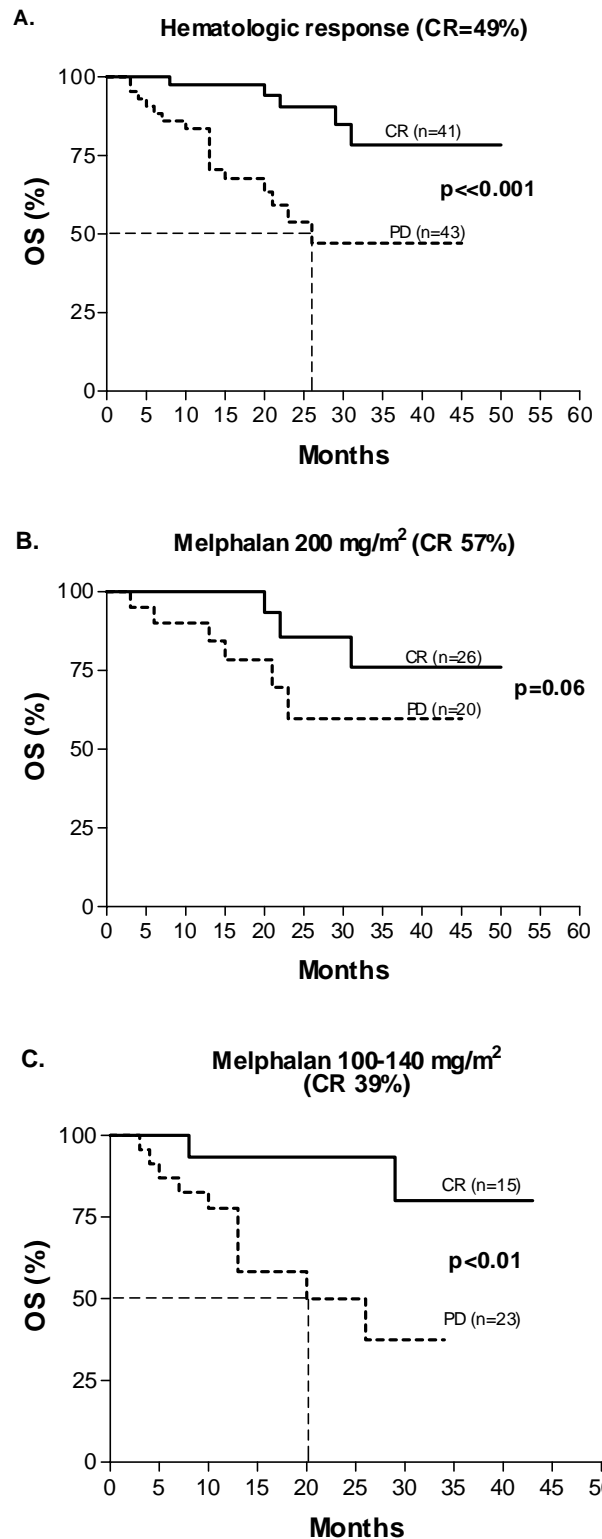


Figure 4. Survival for patients treated with hematopoietic stem cell transplantation and seen in follow-up over a 4-year period is shown according to response of the clonal plasma cell disorder and melphalan dose. Patients with CR experienced significantly better overall survival.

Abbreviations for Table 2 and Figure 4: CR, complete hematologic response by marrow and immuno-fixation of serum and urine (concentrated 10x); PD, persistent clonal plasma cell disease.

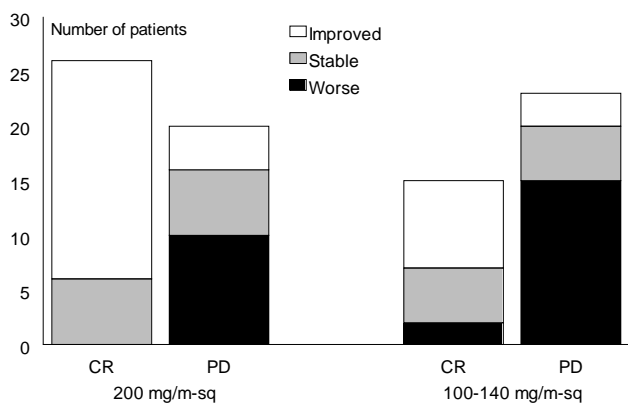


Figure 5. The status of AL organ syndromes was objectively measured in HCT patients in follow-up.^(6,7) Overall responses of involved organs were scored as improved, stable or worse. The distribution of organ responses as a function of dose of melphalan and hematologic responses is shown above. See Figure 4 for definitions of CR and PD.

atrial fibrillation or supraventricular tachycardia, or more typically as hemodynamic instability. In HCT patients with known GI amyloid, the hematocrit should be maintained > 30% and platelets > 50,000 if stool guaiacs are positive. Finally, it is important to note that visceral perforation or rupture due to amyloid occurs rarely in the course of the disease and can occur in the peri-transplant period. Rarely, splenic rupture has occurred but with surgical management patients can survive. Other viscera such as the esophagus or bowel can also perforate and present life-threatening challenges.⁽²⁹⁾

Significant amyloid involvement of the tongue or lungs can pose unusual problems with respect to HCT and, when advanced, becomes a contraindication to the procedure. The potential for airway compromise exists in patients with macroglossia, enlarged submandibular glands and dysphagia, particularly during the toxic period of HCT when mucositis and thrombocytopenic bleeding in the oropharynx may occur. Patients with pulmonary amyloid and symptomatic pleural effusions that require repeated thoracentesis to maintain adequate pulmonary function are not optimal candidates for HCT.

The management of problems related to intravascular volume and hypotension is a critical aspect of the care of AL transplant patients. Nephrotic syndrome causes salt-avidity and hypoalbuminemia, often leading to significant edema. The risk of over-diuresis, however, may be greater than the risk of some peripheral edema in the setting of clinical euolemia. Nevertheless, intravenous fluids for medications should not contain salt whenever possible and maintaining a diuresis concurrently with melphalan administration and stem cell infusion is reasonable, although even mild intravascular volume-depletion may exacerbate nausea and emesis; therefore, limited hydration and cautious diuresis are recommended.

Since a major factor causing pulmonary and peripheral edema is hypoalbuminemia, albumin infusions should be used throughout the treatment period to maintain a level greater than 2 g/dl. Volume-depletion, bleeding, sepsis, SIRS and hypoadrenalism are the most likely causes of hypotension in this setting, not autonomic neuropathy. Use of morphine or fentanyl to treat mucositis can affect both blood pressure and urine output, and can complicate acyclovir prophylaxis since acyclovir toxicity will escalate (particularly CNS toxicity) in the setting of reduced renal perfusion and urine output. Midodrine and fludrocortisone are useful agents to treat orthostasis but do not work reliably in the transplant setting. Careful management of volume status will avoid many of the pitfalls associated with hypotension.

Other valuable supportive care measures include use of incentive spirometry, nutritional and protein supplements, and daily psychiatric visits. It is important to remember that AL patients have an orphan disease that is as fatal as the worst of cancers and that health-care providers who express ignorance of AL cause them great anxiety. It is also important to understand that AL patients are far less medically experienced than the average transplant patient. This combination of factors can cause communication difficulties between patients and their families on the one hand and the transplant team on the other. The most useful approach in dealing with AL transplant patients is to validate their concerns and deal directly with their problems.

In patients with dominant cardiac amyloid with uncomplicated symptomatology, preserved left ventricular function usually assures diuretic responsiveness, although in some instances pre-transplant catheterization with a fluid challenge may provide a useful confirmation of the patient's functional capacity in that regard. Mainte-

Table 4. Frequency of grade ≥2 toxicity according to melphalan dose after hematopoietic stem cell transplantation for amyloidosis.*

Toxicity	200 mg/m** % (n)	100 mg/m*** % (n)
Nausea or vomiting	83 (19)	52 (14)
Diarrhea	65 (15)	48 (13)
Mucositis	91 (21)	37 (10)
Pulmonary edema	35 (8)	26 (7)
Peripheral edema	48 (11)	15 (4)
Non-gastrointestinal bleeding	71 (4)	0 (0)
Gastrointestinal bleeding	22 (5)	7 (2)
Hepatic	13 (3)	22 (6)
Renal	35 (8)	19 (5)
Metabolic	35 (8)	7 (2)
Sepsis	26 (6)	11 (3)

* Southwest Oncology Group criteria ** n = 23 *** n = 27

nance of normal electrolyte balance in cardiac patients being diuresed is an obvious requirement. The transplant-related mortality associated with cardiac amyloid is due to sudden cardiac death and to cardiopulmonary failure involving hypotension and hypoxia. Patients rarely, if ever, survive when ventricular arrhythmias or bradycardic episodes occur post-transplant despite the addition of appropriate medications and use of advanced life-support measures. Whether prophylactic anti-arrhythmic agents or devices can decrease mortality remains to be investigated.

The Pathophysiology and Genetics of AL

As a consequence of treating AL with HCT important lessons about the pathophysiology and genetics of the disease have been learned. First, the use of HCT has clearly demonstrated that AL deposits and their effects are reversible if the clonal plasma cell disease is significantly reduced.^(6,15) Second, the clonal immunoglobulin variable region light chain germline genes used by AL plasma cells affect both the organ-system tropism of the disease and post-transplant survival.⁽³⁰⁾

The tempo of recovery of organ function post-transplant is variable but usually takes months to years; some patients improve significantly but do not normalize entirely while others may achieve normal levels of alkaline phosphatase or proteinuria over a 2 or 3 year period. Indeed, although all major amyloid-organ syndromes have been shown to improve, the process of resorption and organ recovery remains to be understood. Serial biopsy studies of involved viscera in recovery have not been performed to any notable extent. However, amyloid scans have clearly demonstrated a diminution in AL deposits post-transplant in patients with significant hematologic responses.^(8,15) In responding patients proteinuria declines, hepatomegaly regresses, gastric atony reverses, splenic dysfunction remits, autonomic and peripheral neuropathies recover and myocardial hypertrophy diminishes.^(6,31)

Performance of HCT clinical trials in AL has also afforded the opportunity to study the genetics of this light chain immunoglobulin deposition disease.^(30,32) B cell development involves the rearrangement of immunoglobulin (Ig) gene segments, conferring on each mature B cell one heavy and one light chain germline variable region gene (V_H and V_L) that subsequently may undergo somatic hypermutation in the germinal centers of lymph nodes.⁽³³⁾ Multiple myeloma clones are post-germinal center clones with somatically hypermutated V_H and V_L genes that remain unchanged through the course of disease.⁽³⁴⁻⁴⁰⁾ AL is similar to myeloma except that the plasma cell burden is lower, fibrillar depositions are found and λ light chains predominate (κ to λ ratio 1:3).^(3,41) Also, AL patients usually present symp-

tomatically with a dominant involved organ-system. These distinctive features—particularly the organ-system tropism—remain unexplained.

In a recent report the V_H and V_L genes in 14 cases of AL were found to display evidence of hypermutation, adding support to the view that AL clones derive from post-germinal center B cells.⁽⁴²⁾ Recently a similar approach to cloning AL V_L genes was described by using the reverse transcriptase polymerase chain reaction (RT-PCR) with primers specific for V_L first framework (FRI) and 5' constant region (C_L) sequences.^(30,32,43) This line of investigation was pursued in part to test the hypothesis that clonal germline gene utilization influenced the organ-system tropism of AL. The results demonstrated an association between clonal AL V_L germline gene utilization, dominant amyloid-related organ involvement and post-transplant survival, based on data from 62 AL patients treated with either 200 or 140 mg/m² of melphalan over a three and a half year period.⁽³⁰⁾

Clonal AL genes were identified in 39 of 62 instances and were found to be derived from donors of the λI (n=10), λII (n=5), λIII (n=6), λVI (n=11) and κI (n=7) subtypes. The majority of the donor genes (*IGLV6S1*, *DPL5*, *DPL2*, *DPL23* and *LFVK431*) appear in the normally expressed repertoire less than 5% of the time, suggesting an intrinsic propensity to form amyloid under certain conditions. Evidence was found for a link between germline gene utilization and organ-tropism. Patients whose clones derived from the λVI *IGLV6S1* donor uniformly presented with dominant renal involvement while those with other V_λ or unknown donors often had dominant cardiac or other organ involvement, particularly patients whose clones derived from the λI *DPL2* donor (4 of 5 patients with dominant cardiac and > 2 systems involved). In addition, both early (< 3 months) and overall post-transplant survival were significantly better in λVI *IGLV6S1* patients compared to patients with other V_λ donors (**Figure 6**).

Indeed, with respect to survival, the significance of dominant renal amyloid on the one hand and dominant cardiac or multisystem amyloid on the other has been appreciated previously by numerous investigators.⁽³⁻⁵⁾ Patients with dominant renal disease usually live longer than those with dominant cardiac involvement. Therefore, to some degree, the identification of the association between λVI *IGLV6S1* germline gene use, dominant renal involvement and improved post-transplant survival represents a refinement of this earlier observation. Nevertheless, the implications of these findings are of interest and these associations among Ig V_L gene utilization, organ-system tropism and post-transplant survival may be of use in refining patient selection.

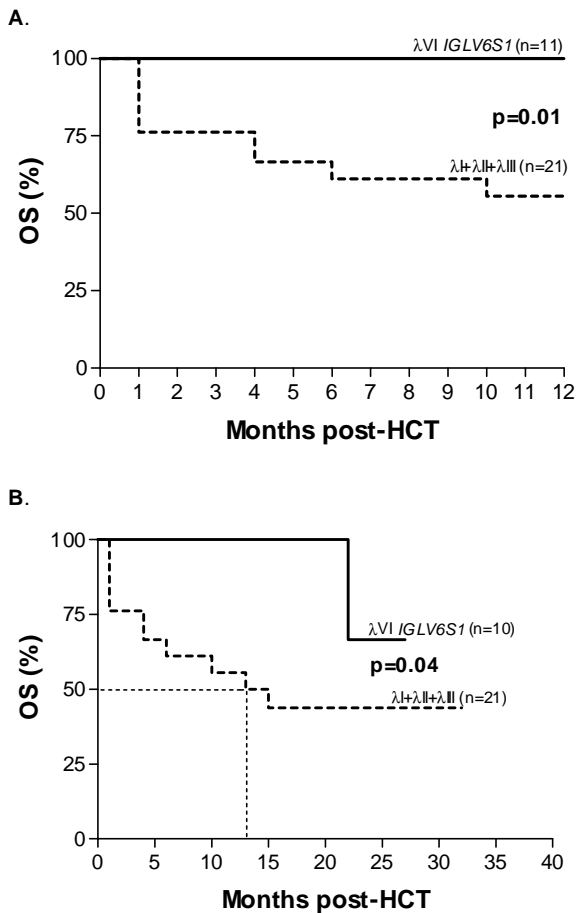


Figure 6. These Kaplan-Meier plots depict the impact of Ig V_{λ} germline gene use on post-transplant survival in AL patients followed for a median of 16 months post-transplant (range, 3-38). In 6A, a significant difference is appreciated between the early survival of λVI *IGLV6S1* patients versus those whose clones used other V_{λ} donors. Of note, λVI *IGLV6S1* patients, all of whom had predominant renal involvement, had no treatment-related mortality. In 6B, the difference in overall survival between these two groups is also shown to be significant. The median post-transplant survival of patients with other V_{λ} donors is 13 months. Of the initial 11 λVI *IGLV6S1* patients, two died, one at 22 and the other at 31 months post-autograft. The latter patient, a 31-year-old woman, died as the result of graft-versus-host disease on day +65 of an HLA-identical sibling allotransplant and is not included in B. Both of these patients had asymptomatic cardiac involvement at baseline.

Use of Blood Stem Cells

Mobilization and leukapheresis

The optimal method for mobilizing stem cells in AL patients remains undefined. An important lesson learned from the use of blood stem cells is that AL patients are at risk of significant morbidity during stem cell mobilization and collection no matter the approach. Other lessons include the confirmation that stem cells can be mobilized and can engraft (including highly purified CD34-selected cells) despite marrow amyloid depositions, and

that they contain contaminating clonotypic cells.⁽³²⁾

Deaths have been reported during mobilization of patients with symptomatic cardiac amyloid or multisystem disease, both at centers employing moderate doses of cyclophosphamide (eg, 2.5 g/m²) and those using G-CSF alone.^(6,44) During mobilization with G-CSF (16 ug/kg/d x 5d), we and others have rarely observed a sometimes fatal though unexplained syndrome associated with progressive hypoxia and hypotension unresponsive to supportive measures; it can occur in patients without cardiac involvement and may be due to a combination of the effects of G-CSF, leukapheresis-activated platelets, pulmonary shunting or cytokine release.⁽⁷⁾ Less toxic complications of mobilization and leukapheresis can also occur in nephrotic patients who become edematous, requiring judicious diuresis and occasional albumin infusions while, more seriously, rapidly accumulating pleural effusions and flash pulmonary edema can occur in patients with both renal and cardiac involvement. In addition, to minimize citrate toxicity in neuropathic and cardiac patients, it often helps to use heparin for anticoagulation during leukapheresis. With these concerns in mind, a reasonable approach to the collection period is to follow a patient's systolic blood pressure, room air oxygen saturation, weight, and heart and lung exam and to cease mobilization and leukapheresis in patients who experience decreases in oxygen saturation to less than 95%, fail to improve promptly with diuretics, or demonstrate unexplained declines in systolic blood pressure to less than 90 mm Hg. There is a morbidity rate of about 15% in AL patients during mobilization and leukapheresis, and collections may need to be interrupted because of excessive weight gain, worsening edema or decreased oxygen saturation.⁽¹³⁾

In the initial cohort of AL patients, the numbers of circulating CD34+ cells pre-leukapheresis in patients who had previously received more than 200 mg of oral melphalan pulse therapy were significantly lower than in patients who had previously received less or no melphalan.⁽⁶⁾ However, there were no significant differences between these groups with respect to CD34+ cells collected on individual days or in toto. Overall a median of 8.9 x 10⁶ CD34+ cells/kg were collected in 2 or 3 large-volume leukaphereses (range 2.9-25.4) in this cohort. In another group randomized to receive G-CSF (10 ug/kg/d) or serial GM- then G-CSF, more CD34+ cells and colony-forming units granulocyte-macrophage (CFU-GM) were collected on the first day of leukapheresis (day 5) in the G-CSF alone group and on the third day of leukapheresis (day 7) in the GM+G-CSF group.⁽¹³⁾ However, similar numbers of CD34+ cells and CFU-GM were collected overall. Of note, patients with no prior oral melphalan therapy had significantly more CD34+ cells collected than patients who had received

prior oral melphalan, a median of 4.9×10^6 (1.9-10.4) versus 2.6×10^6 CD34+ cells/kg (0.5-7.0) ($p=0.017$).

Engraftment and use of CD34-selected cells

Two-thirds of these patients had amyloid identified in the bone marrow by Congo red staining, demonstrating that AL depositions do not impair stem cell mobilization.^(6,13) Engraftment employing unselected stem cells was typical for the CD34 doses used, with the median time to neutrophil and platelet recovery occurring by days 10-11 and 14, respectively, demonstrating that AL depositions do not interfere with engraftment. In addition, 15 AL patients were enrolled in a trial in which CD34 selection was attempted from G-CSF mobilized blood stem cells.⁽⁴⁵⁾ Using the Isolex device (Nexell, Irvine, CA), we obtained a median yield and purity of 42% and 85%, respectively, and median CD34+ cell dose per kg of 4.1×10^6 . Twenty-seven percent of patients (4/15) failed to achieve the required dose of CD34-selected cells/kg ($\geq 2 \times 10^6$) in 2 selections; these patients (whose median age was 62 (56-70)) received either marrow or additional stem cell collections to support therapy. Of note, in patients receiving CD34-selected stem cells myeloid recovery was equivalent to that seen with unselected cells but lymphoid recovery was significantly delayed.⁽³²⁾ Median CD4+ T cell concentration in peripheral blood in the third week of recovery was 840/ μ L (581-1151) in unselected and 76/ μ L (11-191) in CD34-selected patients, respectively.⁽³²⁾ Opportunistic infections were observed in several of the CD34-selected patients. The CR rate was not significantly higher,^(32,45) and hematologic relapse occurred in 1 patient at a year.

Clonotypic contamination

The use of CD34-selected components was based on the finding that clonotypic contamination of stem cells occurred in AL; indeed, recent data indicate that circulating clonotypic cells are commonly found in the blood of AL patients.⁽⁴⁶⁾ Methods based on the polymerase-chain reaction (PCR) were used to assess stem cells for contamination. First, employing the Ig V_L gene sequences described earlier, primer-pairs were created for each patient by identifying the unique *CDR1* and *CDR3* sequences and designing primers intended to give PCR products of a specific size.⁽³²⁾ Two different PCR techniques were employed to assess stem cell collections for contamination: RT-PCR in which stem cell cDNA was used as substrate in single-round *CDR1-CDR3* or nested PCR; and a less-sensitive method of limiting-dilution PCR analysis in which stem cell DNA equivalent to 200,000 cells was used in log-dilutions allowing quantitative estimates to be obtained.⁽⁴⁷⁾

These approaches were used to evaluate clonotypic contamination in 73 stem cell collections mobilized with

G-CSF from 39 AL patients. All collections were found to be positive for clonotypic cells by RT-PCR. Quantitative PCR was performed with multiple log- and half-log-dilutions of DNA and 6 to 10 replicate PCR at each dilution. A Poisson analysis was then performed with the MAXLIKE program (gift of F. Cremer) by using the number of positive and negative lanes at each dilution as entry data. A maximum likelihood estimate was calculated, converted to a percentage and used to estimate the number of clonotypic cells per kilogram patient weight per collection. Thirty-eight percent (28/73) of the collections displayed no detectable clonotypic cells by this DNA-based method. The median percent clonotypic cells in the 45 contaminated collections was 0.006 (range, 0.00023-1.0), and the median number of clonotypic cells detected for all 73 specimens was 1×10^4 per kilogram (range, 0-390).

Thirty-six patients were transplanted with blood stem cells only (3 patients received stem cells plus bone marrow) but only 30 patients were evaluable at 3 month follow-up (5 died prior to 3 months, and 1 died at 10 months without follow-up). Of these 30 patients, 4 of 9 with PCR-negative collections achieved CR as did 10 of 21 with PCR-positive collections, a non-significant difference ($p=1.0$, Fisher's exact test). However, when one looks at overall survival at a median of 2 years as a function of clonotypic contamination in all 36 patients supported with blood stem cells, 9 of 9 patients with PCR-negative stem cells are alive as compared to 9 of 17 patients with less than 0.01% contamination and only 3 of 10 patients with 0.01% or more contamination ($p<0.01$, Chi-square). The significance of clonotypic contamination would appear to be linked to features of the underlying plasma cell disorder, although the question clearly remains as to whether the relapse of clonal disease years after transplant may be due to infused clonotypic cells with proliferative capacity and a variable growth rate.⁽⁴⁸⁾

Future Progress

The development of clinical research based on these results is an area worthy of effort. A major goal should be to improve the outcome for better risk patients in the poor risk groups (Table 3); in order to do so, it may be useful to investigate increased stem cell dosing, modified melphalan dosing, combination chemotherapy with melphalan, and improved supportive care measures. With respect to the last, there may be a role for post-transplant high-dose G-CSF to minimize the occurrence of SIRS and for prophylactic use of anti-arrhythmic agents such as amiodarone or implanted defibrillators. Treatment on well-designed phase II trials is appropriate, as would be the use of conventional oral chemotherapy if such trials are not available. To expose the better risk

patients of this poor risk group on a routine basis to the toxicities of dose-intensive therapy is far less acceptable. Furthermore, if major centers collaborated, phase III trials could be designed for these patients in order to compare the efficacy of HCT versus standard oral chemotherapy.

For good-risk patients HCT is a standard therapy, despite claims that eligibility for HCT is an independent favorable prognostic factor. Indeed, in a recent analysis by experienced investigators, the conclusion was drawn that AL "patients eligible for [HCT] comprise a good risk population who have a superior median survival with standard chemotherapy (45.6 months)."⁽⁴⁹⁾ It is to be hoped that future descriptions of this "good risk" historical cohort will include the frequency and effect of progressive AL disease in such patients. Evidence that weighs against these claims and the position that a phase III trial is indicated in good risk patients include (1) the fact that two large phase III trials employing oral chemotherapy in AL failed to demonstrate complete hematologic responses and improvements in amyloid organ syndromes with a high frequency, (2) the documented superiority of dose-intensive approaches in multiple myeloma, and (3) the high complete hematologic response rate and frequency of improvement of amyloid organ syndromes observed with HCT in AL. Although much room for discussion remains, it may be more reasonable to conduct innovative phase II trials for this good risk group with the objective of increasing the CR rate. If CR rates in excess of 70% should be attained, they would likely be associated with curative outcomes.⁽⁵⁰⁾ Approaches worth investigating include the use of pulse steroids in the early post-transplant period as well as reserving a dose of stem cells for a second HCT in the event of persistent disease or relapse.

Finally, perhaps the foremost remaining challenge is the development of therapeutic approaches for patients with advanced cardiac or hepatic AL amyloidosis. Strategies that combine solid organ and stem cell transplantation are mandated by the clinical need. Numerous patients in the US and UK have received stem cell transplants after cardiac allografts and have done well; in fact, recovery, improved well being and extended survival appear to be the rule in these patients (McGregor C, Falk R, Comenzo R, Gertz M, Hawkins P, Apperley J; unpublished observations). Whether patients with hepatic or hepato-renal allografts will do as well is unknown; in any event, persuading transplant surgeons of the merit of such approaches remains a challenging prospect.

In summary, progress has been made in high dose therapy for the treatment of primary systemic amyloidosis; such therapy is effective at reversing the deposition disease but is not without risk. Guidelines have been

developed for patient selection to maximize benefit and minimize treatment-related mortality. Identification of a patient's clonal germline light chain variable region gene may become relevant to patient selection, and development of less morbid approaches to stem cell mobilization and collection would be useful. While there is room for discussion regarding the design of future therapeutic trials, it is reasonable to attempt to improve the complete response rate for good risk patients by continuing efforts on the phase II level. Attempts to improve outcomes for patients with symptomatic cardiac or advanced multisystem disease may require serial solid organ and stem cell transplantation as well as the development of less toxic approaches by using lower doses of melphalan, improved supportive care measures and specific organ-system prophylaxis. If outcomes can be improved, issues related to clonotypic contamination of stem cells will need to be revisited.

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