Long-term results of a risk-adapted approach to melphalan conditioning in autologous peripheral blood stem cell transplantation for primary (AL) amyloidosis

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Background and Objectives. High-dose melphalan with autologous peripheral blood stem cell transplantation (ASCT) is an effective treatment for systemic primary amyloidosis. This procedure is, however, associated with substantial toxicity and mortality, particularly if the heart is involved. Refined selection of patients suitable for transplantation and personalized adaptation of the doses of melphalan might improve the outcome.

Design and Methods. Twenty-two consecutive patients were selected for age, number of organ systems involved, heart and kidney function, and treated with risk-adapted melphalan conditioning. This was first-line therapy in 81% of cases.

Results. Fifty-five percent of the patients had amyloid involvement of two organ systems, with renal involvement predominant in half. Approximately 70% received full-dose melphalan. Toxicity was manageable and three transplant-related deaths (14%) occurred only in the early phase of the study. The median overall survival was 68 months. The intent-to-treat hematologic response rate was 55% at +12 months (complete, 36%; partial, 19%), which was accompanied by organ responses in 75%. Survival was positively influenced by: (i) hematologic response at +3 months (complete+partial responses 55%, median not reached, more than 108 months; no response, median 17 months) (p=0.001); (ii) amyloid involvement of a single organ system (p=0.016). Prolonged follow-up demonstrated that remissions are durable, but relapses may occur as 4 of 12 responsive patients (33%) relapsed, three from complete response, between +30 to +38 months.

Interpretation and Conclusions. The present risk-adapted approach produced acceptable toxicity and peri-transplant mortality with prolonged survival in responsive patients. Additional therapy should be considered if no hematologic response is observed at +3 months after ASCT.

Key words: primary amyloidosis, autologous peripheral blood transplantation, AL amyloidosis.

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Systemic immunoglobulin light-chain (AL) amyloidosis is an uncommon plasma cell disorder. It is a progressive disease that is fatal in patients who do not respond to chemotherapy.[1] A small percentage of monoclonal plasma cells in the bone marrow (median 7%) secrete light chains [most frequently of the λ isotype (λ/κ ratio, 3:1) and derived from a restricted repertoire of variable region germline gene segments].[2] The light chains accumulate in blood vessel walls and interstitial spaces[3] and with time, if the production of the amyloidogenic light chain cannot be slowed, amyloid progresses involving multiple systems and eventually causing death from heart or other end organ damage. The current therapy of amyloidosis is aimed at eradicating the marrow plasma cell clone since a significant reduction of circulating free light chains[4] is associated with improvement or stabilization of organ function and a longer survival.[5] On the background of superior results with high-dose melphalan and autologous peripheral blood stem cell transplantation (ASCT) in multiple myeloma, a few amyloid centers experimented this therapy in amyloidosis, obtaining unprecedented response rates.[6] However, high-dose ASCT was characterized by severe toxicity and high transplant-related death rates (up to 40% in a multicenter setting).[7] The number of organs involved and the extent of the organ involvement (principally the heart) appear to be the most important parameters predicting toxicity, and careful patient selection with tuning of the melphalan dose according to the theoretical transplantation risk has been recommended.[6] Aware of the intrinsic frailty of patients with systemic AL amyloidosis and with the primary intent to reduce toxicity, an ASCT program based on individually tailored conditioning doses was started 11 years ago in our Institutions. The program was applied to 22 consecutive patients and melphalan dosage was customized accord-

ing to age, performance status, and heart and kidney function. We here describe the long-term results of this experience with risk-adapted-conditioning ASCT.

Design and Methods

Patients

Twenty-two consecutive patients with AL systemic amyloidosis deemed to be transplant candidates were recruited and treated between 1995 and 2002 (median year of treatment: 1998). All patients gave written informed consent to the procedure and the treatment was in agreement with Institutional Review Board requirements. The patients' characteristics are reported in Table 1. Exclusion criteria are shown in Table 2.

Plasma cell dyscrasia study

High-resolution agarose gel electrophoresis and immunofixation were employed on serum and a 24-hour urine sample. The sensitivity of the immunofixation was established in our laboratory as 10 mg/L.[8] By means of this highly-sensitive technique, 97% of the AL patients have monoclonal component either in serum and/or urine.[9] The percentage of bone marrow plasma cells was evaluated from morphologic assessment of a marrow aspirate and/or biopsy.

Mobilization and harvesting of peripheral blood stem cells

No induction therapy was given. Peripheral blood $CD34^+$ stem cells were mobilized by cyclophosphamide (3-5 g/m^2) plus recombinant human granulocyte colony-stimulating factor (filgrastim) (5 μg/Kg/day) in five patients and filgrastim alone (10 μg/Kg/day, divided in two doses) in the other 17 cases. The target number of $CD34^+$ cells was 7×10^6 per kg of body weight, with a minimum of 3.5×10^6. Apheresis were performed in an inpatient setting, with assistance of an intensive care physician for patients with heart involvement.

Risk assessment and autotransplantation

Pretransplant evaluation included physical examination, complete blood chemistry, 24-hour urine collection, echocardiography, 24-hour electrocardiogram, chest radiography, pulmonary function tests, abdominal cavity ultrasound, coagulation screen with factor X evaluation, fecal occult blood tests, and serology for hepatitis viruses and human immunodeficiency virus. All patients were evaluated by hematology and cardiology specialists at the Amyloid Center in Pavia. In specific cases, patients underwent further investigations to clarify the extent of specific organ involvement or function.

The procedure was performed in transplantation units. The dose of melphalan was established according to the estimated risk (Table 2) and administered in divided doses over two consecutive days (day -2 and -1). Melphalan conditioning was either high-dose (200 mg/m^2), intermediate-dose (140 mg/m^2), or low-dose (100 mg/m^2). Stem cells were reinfused 24 to 48 hours after the last dose of melphalan. Filgrastim (5 μg/Kg/day) was started 48 hours after the stem cell reinfusions and was continued until neutrophil counts were normal. Standard supportive care was given and patients remained in hospital until stable hematologic recovery had been achieved. This was defined as stable

Table 1. Characteristics of the 22 patients with AL amyloidosis before ASCT, their conditioning regimens and the transplant-related mortality.

Characteristic	*Number (%) or median (range)*	*% Abnormal*	*Abnormal value*
Male:female	16:6		
Age (years)	51 (31-65)		
Performance status (ECOG)			
0	10		
1	10		
2	2		
Months from diagnosis	7 (2-34)		
Previous MEL-based therapies			
0	18 (81%)		
< 200 mg	1		
> 200 mg	3		
Involved organs (n°)			
1	10		
2	12 (55%)		
Dominant organ			
Kidney	11(50%)		
Heart	5 (23%)		
Other[a]	6 (27%)		
Creatinine, mg/dL	0.9 (0.7-2.8)	23	>1.4
24-hour urine protein (g)	6.45 (0.1-26)	59	>3
Alkaline phosphatase (U/L)	207 (108-868)	14	>279
Monoclonal plasma cells (%)	10 (2-27)	0	>30
Echocardiogram septal thickness (mm)	12 (7-18)	50	≥12
Ejection fraction (%)	56 (37-77)	9	<50
NYHA class II patients among those with heart involvement (n=10)	3 (30%)		
Monoclonal light chains: κ/λ	1/21		
Monoclonal free light chains exclusively	13 (60%)		
Conditioning regimens			
Low-dose MEL	1 (5%)		
Intermediate-dose MEL	6 (27%)		
High-dose MEL	15 (68%)		
Treatment related mortality	3 (14%)		
Pre stem-cell collection	0		
Stem cell collection	0		
Within 100 days of MEL	3 (14%)		

MEL: melphalan; MC: monoclonal component; [a]Other organs are: soft-tissues (muscle, tongue, submandibular glands), five cases; peripheral/autonomous nervous system, one case.

Table 2. Selection criteria for ASCT in AL amyloidosis and relative melphalan dose.

Low risk (all criteria must be satisfied)	*Intermediate risk*	*Not eligible*
<60 years Organs involved ≤2 Ejection fraction >50%	60-65 years Serum creatinine ≥1.5 mg/dL Compensated heart involvement (NYHA II)[c]	>65 years Organs involved ≥3 NYHA III or IV
Serum creatinine ≤1.4 mg/dL	Performance status 2	Serum bilirubin >2 mg/dL
CO lung diffusion >50% predicted		CO lung diffusion <50% predicted
Asymptomatic heart involvement[a] Performance status ≤1[b]		·systolic BP[d] <90 mmHg performance status 3
Melphalan dose	*Melphalan dose*	
200 mg/m^2	140 mg/m^2 100 mg/m^2 if NYHA class II	

[a]with evidence of heart involvement at echocardiography; [b]ECOG Performance Status Scale; [c]New York Heart Association classes; [d]BP: arterial blood pressure.

neutrophil (>1.0×10^9/L) and platelet (>20×10^9/L) counts without transfusion support. *Second ASCT*. Patients who underwent a first ASCT with high-dose melphalan with no substantial problems and obtained partial hematologic response at +3 months were considered for a second high-dose transplantation.

Toxicity was evaluated according to the NCI criteria (CTC version 2.0), and performance status according to ECOG/WHO criteria. The patients' scheduled follow-up was physical examination and hematologic/organ response evaluations every 3 months. No maintenance therapy was given after the stem-cell transplant.

No effort was made to compare toxicity, response and outcomes with respect to melphalan dose because the study was not designed with this aim.

Evaluation of hematologic response

A partial response was defined as a reduction of at least 50% of serum and urine monoclonal components; complete response was defined as the disappearance of the monoclonal component in serum and urine by immunofixation. No bone marrow re-evaluation was required. The response had to be maintained for at least 3 months (two consecutive follow-up visits).

Evaluation of organ involvement and response

Organ involvement was evaluated for each patient at study entry. Patients were stratified according to the main organ involved and the number of organs involved according to clinical judgment and by consensus among three different investigators (*VP, GP, GM*). The judgment was based on main clinical manifestations, laboratory data, and ultrasound findings. Briefly, seven organ systems are considered: heart, kidney, liver, soft tissue, intestine, nerve (including both peripheral and autonomous nervous systems), and lung. Kidney involvement was defined as daily proteinuria >0.5 g and/or serum creatinine >1.4 mg/dL. Heart involvement was defined as interventricular septum thickness ≥12 mm [>11 mm in the presence of increased echogenicity (*granular sparkling*) or signs of diastolic dysfunction, ≥13 mm in the case of systemic hypertension], or signs of congestive heart failure (distension of jugular veins, liver congestion and peripheral edema). Liver amyloidosis was documented by hepatomegaly (>2 cm below the costal border) with serum alkaline phosphatase value 1.5 times the upper limit of our institutional value (279 U/L). Peripheral and autonomous nervous system involvement was defined clinically (the presence of carpal tunnel syndrome alone did not constitute evidence of peripheral nervous system involvement), as was soft-tissue amyloid infiltration. In the case of multiple organ involvement, patients were assigned according to the main clinical characteristics. Heart failure was graded according to the NYHA classification.

Organ response criteria were similar to those reported by Gerts *et al.*[10] Briefly, kidney response was defined as a >50% decrease of 24-hr urine protein in the absence of a 25% increase in the serum creatinine concentration or a 25% decrease in creatinine. Heart response was documented by a mean interventricular septum thickness decrease of 2 mm, 20% improvement in ejection fraction, improvement by one NYHA class, or decreased use of diuretics with decreased heart echogenicity. Liver response was defined as a decrease of an abnormal serum alkaline phosphatase value by 50%. Soft tissue, peripheral and autonomous nervous system improvements were assessed clinically. The degree of organ involvement was classified at each follow-up as improved, stable or worsened on the basis of instrumental and biochemical parameters.

Statistics

Survival curves were plotted according to the Kaplan-Meier method and the difference in survival tested for significance with the log-rank test. Differences between 24-hour urine protein loss before and after transplantation in patients with kidney involvement who attained hematologic response were tested using a Wilcoxon's matched pair test.

Results

Table 1 presents the clinical characteristics of the patients enrolled in the study, their stratification and

Table 3. Clinical characteristics, responses (+12 months) and outcomes of the 22 patients who underwent ASCT.

Patient	Age	Alive-Dead	ASCT conditioning	Predominant organ involved	Secondary organ involved	Hematologic response[a]	Organ response[a]	Hematologic relapse	Follow-up
1	39	Alive	HDx 2	Heart	Soft tissues	CR	+		+108
2	47	Alive	HDx 2	Kidney	–	CR	Worsened[b]		+98
3	31	Alive	HDx 2	Soft tissues	–	CR	+		+68
4	52	Alive	HD	Kidney	–	PR	+		+73
5	44	Alive	HD	Kidney	–	CR	+		+52
6	44	Alive	HD	Kidney	Heart	CR	+	+30	+91, dialysis
7	52	Alive	HD	Kidney	–	PR	Stable		+73
8	51	Alive	HD	P/ANS	Kidney	PR	Stable	+38	+79, progression
9	53	Alive	ID	Heart	–	CR	+	+37	+76
10	56	Alive	ID	Kidney	–	NR	Stable		+47
11	55	Alive	ID	Soft tissues	–	CR	+		+47
12	50	Dead	HD	Kidney	Heart	NR	Worsened[b]		-18
13	45	Dead	HD	Soft tissues	Kidney	NR	+		-13
14	52	Dead	HD	Soft tissues	Heart	PR	+		-36
15	43	Dead	HD	Heart	–	NA	NA		TRD
16	51	Dead	HD	Soft tissues	Heart	NR	Worsene[d]		-17
17	49	Dead	HD	Kidney	Heart	NA	NA		TRD
18	58	Dead	HD	Kidney	Liver	NA	NA		TRD
19	53	Dead	ID	Kidney	–	CR	+	+36	-68
20	58	Dead	ID	Kidney	GI tract	NR	Worsened		-13
21	65	Dead	ID	Heart	P/ANS	NA	NA		-8
22	49	Dead	LD	Heart	Kidney	NR	Worsened[b]		-23

[a]at +12 months after ASCT; [b]progressive renal insufficiency with subsequent dialysis. ASCT: autologous stem cell transplantation; HD: high-dose melphalan; ID: intermediate-dose; LD; Low-dose; P/ANS, peripheral/autonomous nerves; GI: gastrointestinal; CR: complete response; PR: partial response; NR: no response; NA: not applicable because of death before evaluation (+12 months); +, improved; TRD, treatment related-death.

their doses of melphalan conditioning. All patients completed treatment and none was lost to follow-up. Fifty-five percent had clinical involvement of two organ systems and several patients had evidence of organ dysfunction. Ventricular ejection fraction was abnormal in only two cases. It is recognized that this parameter is of limited value in heart amyloidosis.[10] Since amyloid patients are at an increased risk of complications during stem cell harvesting,[6] a maximum of three procedures were allowed. The target number of $CD34^+$ cells (7×10^6 $CD34^+$/Kg) was harvested from 60% of the patients. The median number of $CD34^+$ progenitors collected was 7.1×10^6/Kg (range 4.2-18). The procedure was generally well tolerated. Reinfusions occurred in the absence of severe complications except in two patients (both of whom had grade 4 cardiovascular collapse, probably dimethylsulfoxide-related, which was successfully treated with atropine). Two-thirds (68%) of the patients received full-dose chemotherapy (high-dose melphalan), and Table 3 summarizes responses and outcomes of the 22 patients according to their type of conditioning and clinical characteristics.

Table 4. Frequency of grade >2 toxicities and transfusion needs in the transplanted AL population.

Toxicity (grade >2)	%
Vomiting/diarrhea	36
Mucositis	27
Neutropenic fever	23
Sepsis	14
Liver	14
Non-neutropenic fever	9
Heart	9
Kidney	9
Internal hemorrhage (gastrointestinal or other)	0
Peripheral edema	0
Transfusion components	**Median (range)**
Packed red blood cell units	1 (0-4)
Concentrated platelet units from apheresis	2 (1-3)

National Cancer Institute Common Toxicity Criteria (CTC version 2.0).

Toxicity

Patients with systemic light-chain amyloidosis have both clinical and subclinical organ alterations, which are responsible for increased morbidity and mortality under conditions of stress.[6] Table 4 shows the frequencies of grade 3-4 toxicities observed in the patients and their transfusion needs. The toxicities observed were those typically occurring in ASCT, and they were clustered in patients with cardiac amyloidosis (Table 5). Gastrointestinal toxicity (mucositis, vomiting/diarrhea) was more severe with full-dose conditioning. No bleeding episodes were observed and, in particular, no gastrointestinal tract hemorrhage occurred although the frequency of this complication is reported to be increased in AL patients.[11] Patients were hospitalized for

Table 5. Organ function parameters of patients with predominant heart involvement, at ASCT and at response evaluation (+12 months). Toxicity and treatment-related deaths are reported.

Patient	ASCT conditioning	Toxicity (grade >2)	IVS (mm)/ EF (%) pre-ASCT	IVS (mm)/ EF (%) post-ASCT[a]	NYHA class pre-ASCT	NYHA class post-ASCT[a]
1	HD×2	–	18/56	18/58	I	I[b]
15	HD	Sudden death	18/53	NA	I	NA
9	ID	Heart	16/37	13/55	II	I
21	ID	Febrile neutropenia	18/44	NA	II	NA
22	LD	DIC, febrile neutropenia	18/50	21/58	II	III

[a]at +12 months; [b]decreased diuretic use. ASCT: autologous stem cell transplantation; IVS: interventricular septum; EF: ejection fraction; NYHA: New York Heart Association; HD: high-dose; ID: intermediate-dose; LD: low-dose; NA: not applicable because of death before evaluation of organ responses; DIC: disseminated intravascular coagulation.

a median of 23 days (range 16-41).

Hematologic toxicity. Neutrophil and platelet recovery was observed after a median of 11 (range 9-15) and 12 days (range 9-19), respectively. Grade 4 neutropenia lasted a median of 6 days (range 4-10). Similar reconstitution times were recently reported.[12] Transfusion needs were modest and no major bleeding episodes were recorded.

Kidney. Treatment-related toxicity was observed, despite achievement of a hematologic complete response, in one patient who underwent a second high-dose treatment and whose serum creatinine was 1.4 mg/dL (upper reference interval: 1.2 mg/dL) before the first transplant (Table 6, #2).

Heart. Five patients with predominant amyloid heart involvement were treated. The clinical characteristics of this group are reported in Table 5.

Peri-transplant mortality (100 days). The incidence of death in the peri-transplant period was 14% (3 of 22 patients) (Table 1). Sudden death occurred during the procedure in one patient with predominant heart involvement (Table 3, #15), one patient with liver amyloidosis died of hepatic veno-occlusive disease (Table 3, #18) and one patient died of cytomegalovirus infection (predominant kidney involvement, Table 3, #17). All three deaths occurred in the initial period of applying the protocol (one in 1995 and two in 1996).

Survival

Eleven of 22 patients (50%) died after a median follow-up of 13 months (range: 1-68 months, 14% in the peri-transplant). With the exception of the three treatment-related toxic deaths, all other patients died of disease-related causes.

The median follow-up of the entire population was 47 months and that of the 12 patients alive at time of analysis was 73 months (range 47-108). The median survival of the entire cohort was 68 months (Figure 1).

Table 6. Organ function parameters of patients with predominant kidney involvement, at ASCT and at response evaluation (+12 months).

Patient	ASCT conditioning	Creatinine pre-ASCT (mg/dL)	Creatinine post-ASCT[a] (mg/dL)	Daily (g/24h) proteinuria pre-ASCT	Daily (g/24h) proteinuria post-ASCT[a]
2	HD×2	1.4	2.3[b]	26	28
4	HD	0.6	0.8	6.7	0.5
5	HD	0.8	1.0	13.5	6
6	HD	0.9	0.9	14.7	6
7	HD	0.7	1.1	6.2	6.1
10	ID	0.7	0.7	16	10.3
12	HD	0.8	0.8[b]	7.16	45
19	ID	0.7	0.5	23	8.1
20	ID	1.6	7.9[b]	12	6.8

[a]+12 months; [b]progressive renal insufficiency with dialysis. ASCT: autologous stem cell transplantation; HD-MEL: high-dose melphalan; ID: intermediate-dose.

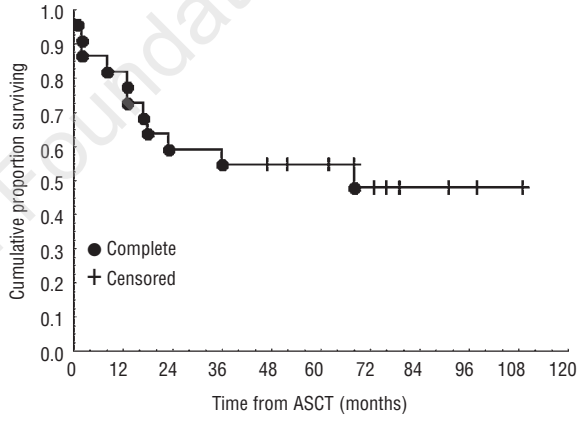

Figure 1. Survival analysis (Kaplan and Meier, log-rank analysis) of the 22 patients with AL amyloidosis following ASCT. The overall median survival was 68 months.

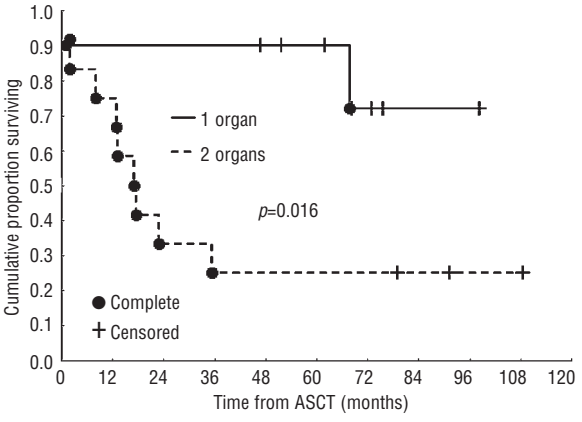

Figure 2. The number of organs involved at the time of ASCT influences survival. When patients are divided according to the number of organ/systems involved (one or two), patients with more advanced disease have poorer outcomes (Kaplan-Meier, log-rank analysis) after ASCT, although long-term survival may be observed in some cases.

Patients with a single organ involved had a better survival (p=0.016, log-rank test, Figure 2). The majority (80%) of the patients with single organ involvement are alive (five with kidney involvement, two with soft tissue involvement and one with heart involvement). By contrast, only a quarter (25%) of the patients with two organs involved are alive.

Hematologic response

At 3 months. Almost half of the patients attained hematologic partial response at +3 months (intent-to-treat partial response rate, 45%; eight of 15 treated with high-dose melphalan and two of five treated with intermediate-dose melphalan). Two patients had a complete response (9%, one treated with high-dose melphalan and one treated with intermediate-dose melphalan), but seven were unresponsive (32%, three treated with high-dose, three with intermediate-dose and one with low-dose melphalan). Three patients were not available for analysis because of early death. Overall, the intent-to-treat hematologic response rate was 55% at +3 months.

At 12 months. The quality of hematologic response improved with time. At +1 year, 60% of patients with a partial response had attained a complete remission and in three out of the six cases (50%), conversion was achieved without a second ASCT. Therefore, just 4 of the 12 responsive patients were in partial remission at 1 year (Table 3, #4, serum monoclonal component changed from to 7.0 g/dL to 3.0 g/dL, #7 from 6.4 g/L to 2.6 g/dL, #8 from 1.7 g/L to 0.8 g/L, #14 from 8.7 to 2.3 g/L).

Lack of hematologic response at +3 months was associated with a poor chance of later improvement, since none of the seven unresponsive patients at +3 months obtained a partial response by +1 year. Thus, taking into account the sustained response of the two patients already in complete response at +3 months, the intent-to-treat hematologic complete response rate was 36%, for an overall response rate (complete + partial responses) of 55%.

Hematologic response and survival

To test whether early response was predictive of improved survival, the outcome of patients with any hematologic response at +3 months (partial + complete responses) was compared with that of non-responsive patients. The outcome analysis indicated that the estimated survival curves differ significantly (p=0.001, log-rank test) (Figure 3). The median survival of non-responders was only 17 months, with just one patient still being alive at +47 months (Table 3, #10). The median survival of responders had not been reached at the time of this analysis (Figure 3).

Response to ASCT translated into extended survival even in the patients with predominant heart involvement (two of five patients alive at +108 and +76 months) (Table 3, #1, #9). However, relapses occurred. Four of the 12 responsive patients (33%, i.e. 18% of the total transplanted population) had a recurrence or >50% increments of the monoclonal component by approximately 3 years after ASCT: three relapsed from complete responses at +30, +36 and +37 months (Table 3, #6, #19, and #9, respectively), and one from a partial response, at +38 months (Table 3, #8).

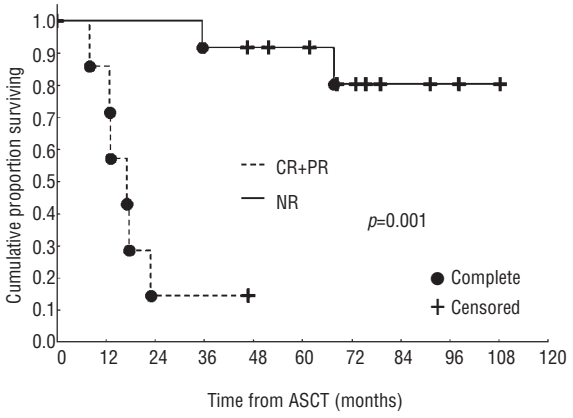

Figure 3. Early hematologic response and extended survival after ASCT. Patients were divided at +3 months according to hematologic response (complete and partial vs no response). Early hematologic response was associated with significantly improved survival times (log-rank analysis, p=0.001).

It should be noted that hematologic relapse did not translate into organ dysfunction in all cases: for example, a patient with cardiac amyloidosis who achieved a complete response and then had hematologic relapse (monoclonal component detected by immunofixation only) 37 months post-ASCT shows no signs of cardiac progression so far (+62 months) (Table 3, #9).

Organ responses at 12 months

Overall, the intent-to-treat organ response rate was 45% (Table 3). Hematologic response (complete + partial responses) led to organ improvement in 9 of 12 cases (75%), stabilization in three, and no organ response in one patient with a complete hematologic response (treatment-related progressive renal failure after the second high-dose melphalan conditioning) (Table 3, #2). Reversal of amyloid syndromes was observed in both heart (Table 5) and kidney patients (Table 6).

In particular, patients with kidney involvement who attained hematologic response had a significant reduction of 24-hour proteinuria (pre-treatment, median 14.1 g/24 h, range 2.5-26; post-treatment, median 6 g/24h, range 0.5-28, p=0.035, Wilcoxon's matched pair test). On the other hand, absence of hematologic response was associated with progression to chronic renal insufficiency in patients with kidney involvement (Table 3, #12, #20, #22), two with abnormal pre-transplant concentrations of serum creatinine (Table 6, #2 and #20).

Discussion

We report the long-term results of an approach to ASCT for primary amyloidosis based on risk-adapted melphalan conditioning. Accurate candidate selection and tuning of the melphalan dose contained toxicity and provided elevated response rates (intent-to-treat analysis: 55% hematologic response, 45% organ response). Hematologic responses were fast, durable, and accompanied by improvements of organ disease in the majority of cases. The absence of any hematologic response at +3 months was indicative of no further improvement and of a negative outcome. Amyloid involvement of more than one organ at the time of transplantation affected survival adversely.

High-dose chemotherapy is highly effective therapy in AL amyloidosis, since response rates to transplantation are consistently higher than 50-60% (30-35% hematologic complete responses).[6,13,14] However, the early use of this procedure in patients selected according to standard transplantation criteria was the cause of substantial complications, and peri-transplant death rates were exceedingly high, particularly in patients with heart involvement.[7,15] In order to contain toxicity, we adopted selective criteria (Table 2) to establish eligibility for transplantation and the best melphalan conditioning doses. The rates of hematologic and organ responses at +12 months were elevated and were obtained with transplant-related mortality (14%) and toxicity rates (Table 4) that match the current standards.[5] Indeed, since deaths occurred exclusively in the early period of using of the protocol, it is possible that accumulating experience will further improve present results in term of toxicity.

Achievement of hematologic response was accompanied by prolonged survival (we document a median survival of more than 108 months among responsive patients), supporting a primary role for ASCT in selected patients. On the other hand, since eligibility *per se* influences survival positively,[16] there are concerns about the real impact of transplantation. Indeed, while there is no reason to doubt that ASCT is superior to melphalan and prednisone,[17] two recently introduced regimens based on the combination of melphalan and dexamethasone[18,19] have produced response rates approaching those of ASCT with low therapy-related mortality. No information is yet available on duration of responses. A multicenter randomized clinical trial testing ASCT (melphalan 140 or 200 mg/m^2 depending on age and clinical status) vs an oral combination of melphalan and high-dose dexamethasone is ongoing in France. Preliminary results[20] (median follow-up for the living patients is 29.3 months) confirmed the efficacy of the melphalan-high dose dexamethasone regimen in a multicenter setting: no statistical differences between the two arms were observed in overall hematologic (approximately 65% in both) and organ responses (ASCT, 52%; melphalan-high dose dexamethasone, 40%), and survival (ASCT, 48.6 months; melphalan-high dose dexamethasone, 56.9 months). On the other hand, the oral regimen was significantly less toxic, with a survival advantage over ASCT when the analysis was limited to centers with little experience due to excessive transplant-related toxic deaths (the transplant-rerated mortalitry of the trial was 24%). The present observation of improved patient management with time (treatment-related toxic deaths were limited to the early phases of applying the protocol) is in line with these results, further highlighting the need to perform ASCT for AL amyloidosis in referral centers.

The long follow-up of the present study showed that responses to ASCT may be durable, with a small number of hematologic relapses from complete remission (three of the eight patients, 36%) during the third year after transplantation. Later relapses were not observed and the median survival of responsive patients has not been reached. Skinner *et al.* in a review of their 8-year experience in ASCT for AL amyloidosis noted a very low incidence of relapse [just 6 of 73 (8%) patients with a complete response at 1 year relapsed at 2 years and there were no later relapses].[13] At present, the low rate of hematologic relapse indicates that ASCT can produce long-term control of the AL clone in many patients. It should be noted that hematologic relapse does not translate into organ relapse if the monoclonal light chain concentration does not reach the concentration needed for amyloid fibril formation (as suggested in one of our patients, Table 3, #9), a value that is individual and cannot be predicted *a priori*.

According to our study, patients with clinical involvement of two organs systems at the time of ASCT had significantly shorter survival times than had patients with single organ dysfunction. This is a consequence of disease extension and aggressiveness, with an impact on the patient's frailty. Moreau *et al.*[7] previously reported an intolerable risk of death (>75%) following high-dose melphalan conditioning in patients who had two or more clinical manifestations, suggesting that this group of patients should not undergo ASCT. Although having two amyloid syndromes remains relevant for patients' outcome (Figure 2 and Table 3), treatment-related toxicity/mortality can be markedly reduced by appropriate adjustment of the dose of melphalan. The new serum biomarkers of cardiac disease, such as NT-proBNP[21] and troponins,[22] could be of relevance in improving ASCT results further in terms of patient selection/stratification, maximal tolerated dose of melphalan and toxicity, as suggested by the Mayo Clinic group.[23] On the other hand, using lower doses of melphalan might translate into inferior response rates, as suggested by the retrospective analyses conducted by

the Mayo Clinic (high dose, approximately 70-65% responders; lower doses, approximately 55%) and Boston University groups (complete response rates: high-dose melphalan, 45%; lower doses, 33%).[13,24] However, the authors acknowledged that these results should be considered with caution, since the two populations differ for prognostic parameters and recognize the need for prospective studies to address this point correctly. We observed a 43% hematologic complete response rate in the seven patients who underwent reduced conditioning (six intermediate- and one low-dose) suggesting that prolonged survival can be achieved in this unfavorable prognostic group too (two patients are alive, one in complete remission, at +47 and +76 months from ASCT, Table 3, #11, #9, respectively). It is worth mentioning that none of the seven patients treated with reduced conditioning died of toxic death in the peritransplant period. Thus, it appears that ASCT with reduced conditioning is a reasonable option for AL patients who can not tolerate high-melphalan doses, and that despite reduced response rates, this approach allows otherwise ineligible patients to undergo a therapeutic option that can be compatible with prolonged remission.

A double high-dose melphalan course was used in three patients who attained hematologic partial response after the first transplant (Table 3). These patients then had hematologic complete responses and prolonged event-free survival (Table 3). In a study by Comenzo *et al*.[25] double intermediate-dose ASCT could be completed in a small fraction of cases (5 of 27 patients). Given the characteristics of the AL amyloidosis population, it is possible that this strategy may have limited feasibility because of cumulative organ toxicity (one of our patients, who presented with reduced pre-transplant renal function, developed treatment-related toxicity leading to dialysis, despite hematologic complete remission, Table 3, #2). However, the double procedure may prove very efficacious: all three double-transplanted patients (who received high-dose melphalan twice) are alive, in hematologic complete remission, with extended event-free-survival (Table 3, #1-3).

The close follow-up we adopted in our series of patients (every 3 months) allowed us to demonstrate that hematologic responses are usually fast, and the time-point at 3 months post-transplantation is usually adequate to test for response. Indeed, only one of the patients with no hematologic response at +3 months is still alive (Table 3, #10). AL amyloidosis is a progressive disease and the time to initiate alternative therapy is critical in cases of ASCT failure. It seems reasonable to wait 3 months for a hematologic response, and many patients are likely to be sufficiently fit to undergo further treatment at this time. Drugs acting through alternative pathways such as high-dose dexamethasone, alone[26] or in combination with thalidomide,[27] are among the subsequent treatment options.

In conclusion, we show that the present approach to ASCT for AL amyloidosis is feasible and effective. This therapeutic strategy produces elevated response rates with acceptable toxicity when candidates are carefully selected. Responses are fast, durable, and translate into reversal/stabilization of amyloid syndromes in the majority of cases. In order to obtain maximal benefit from transplantation with low toxicity, patients should be treated early in the course of disease, when there is a higher chance of organ function recovery. Early diagnosis is, therefore, crucial, as is close follow-up in order to treat unresponsive cases promptly.

VP designed and performed research, analyzed data and wrote the paper; GP performed research, analyzed data and revised the paper; SS, MB, MDN, LO, MM and LB performed research; AMG performed research and analyzed data; GM designed and performed research and revised the paper. The authors declare that they have no potential conflict of interest.

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