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Autografting

Thiotepa, busulfan, cyclophosphamide (TBC) and autologous hematopoietic transplantation: an intensive regimen for the treatment of multiple myeloma

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Summary:

The study was designed to evaluate the efficacy and safety of an intensive, tri-alkylator conditioning regimen, consisting of thiotepa, busulfan and cyclophosphamide (TBC), prior to autologous hematopoietic cell transplantation in patients with multiple myeloma (MM) and to analyze factors associated with outcome. One hundred and twenty patients with MM received high-dose chemotherapy with TBC followed by autologous bone marrow ($n = 24$) or peripheral blood stem cell (PBSC) transplantation ($n = 96$). Fifty-four patients had chemosensitive disease and 66 had refractory disease at the time of transplantation. The overall response rate was 81% and the complete remission (CR) rate was 26%. Patients with chemosensitive disease had a CR rate of 52% *vs* 5% for patients with refractory disease. Multivariable analysis determined disease status at transplant as the factor most likely associated with long survival. Estimated median survival was 48, 35 and 9 months for patients with chemosensitive, primary refractory or disease in refractory relapse, respectively. Short interval from diagnosis to transplant among patients with primary refractory disease and younger age were also favorable prognostic factors for survival. Patients with refractory disease pre-transplant who achieved remission criteria rapidly after treatment had a worse outcome than the slow responders. Treatment-related mortality with the introduction of PBSC and better supportive care was 4.8%. In conclusion, TBC is an effective and relatively well-tolerated intensive conditioning regimen in patients with MM. A more favorable outcome was observed in patients with chemosensitive disease and with early treatment for primary refractory disease. TBC merits further study in these subgroups and comparison with alternative regimens in prospective studies is warranted. *Bone Marrow Transplantation* (2001) **27,** 821–828.

Keywords: multiple myeloma; autologous transplantation; response kinetics

High-dose therapy followed by autologous bone marrow or peripheral blood stem cell transplantation has emerged as an effective treatment for many patients with multiple myeloma. Standard chemotherapy results in a complete remission (CR) rate of 5%, a median survival of 3 years, and 10% of patients surviving longer than 10 years.[1,2] Studies have shown that survival is improved with high-dose chemotherapy.[3–17] Disease status prior to transplant and time to transplant have been identified as very important prognostic factors for survival.[12–15] Age, β2-microglobulin level, and abnormalities of chromosome 13 have also been shown to be predictive.[3–19]

The kinetics of response after conventional chemotherapy (eg the time to achieve partial or complete remission) has been reported to be an important prognostic factor for treatment outcome by some, but not all investigators.[20–22] The kinetics of response after high-dose chemotherapy has not been thoroughly investigated and its prognostic significance remains unknown. In this study we report our long-term experience with a high-dose tri-alkylator regimen consisting of thiotepa, busulfan and cyclophosphamide for the treatment of multiple myeloma. The aim of the analysis was to define prognostic factors for survival as well as to determine if kinetics of response were important in prognosis.

Patients and methods

Eligibility criteria

Patients were eligible for the study if they had symptomatic myeloma, stage II or III by Durie criteria.[23] Patients were required to be younger than 70 years with a Zubrod performance status $\leqslant 2$, left ventricular ejection fraction $\geqslant 50\%$

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with no uncontrolled arrhythmia, adequate pulmonary function, serum creatinine $\leqslant$2 mg/dl, serum bilirubin $\leqslant$2 mg/dl and no evidence of chronic liver disease. The study was approved by the University of Texas MD Anderson Cancer Center Institutional Review Board and all patients provided written informed consent according to institutional guidelines.

Peripheral blood stem cell/Bone marrow collection

High-dose chemotherapy was supported by autologous peripheral blood stem cells in 96 patients and by autologous bone marrow in 24 patients. Mobilization chemotherapy consisted of cyclophosphamide alone, cyclophosphamide and etoposide combination[24] or fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (hyperCVAD)[25] followed by growth factor stimulation. Apheresis was performed with the Cobe Spectra continuous flow blood cell separator (Cobe, Lakewood, CO, USA) using standard techniques. The target dose was at least 2×10^6 $CD34^+$ cells/kg and patients were allowed to have bone marrow harvest to supplement peripheral blood stem cell collection if this target was not reached.

Twenty-four patients had autologous bone marrow harvests using conventional techniques, of whom 11 patients underwent B cell purging with anti-CD19 monoclonal antibody and immuno-magnetic beads using previously described techniques.[14]

Preparative regimen and supportive care

The preparative regimen consisted of thiotepa 250 mg/m^2 over 4 h given intravenously on days −9, −8, −7; busulfan 1 mg/kg given orally every 6 h on days −6, −5, −4 for a total of 10 doses; cyclophosphamide 60 mg/kg intravenously over 1 h on days −3, −2. Mesna 10 mg/kg was administered prior to the first dose of cyclophosphamide and then at 60 mg/kg/day by continuous infusion for 48 h. Since November 1994 patients were enrolled on a study investigating cyclosporine for induction of autologous graft-versus-host disease. Forty-nine patients received cyclosporine starting on the day of stem cell infusion at a dose of 1–2 mg/kg per day initially by continuous intravenous infusion and then orally for 28 days as previously described.[26]

All patients were treated in private rooms. The prophylactic antibiotic regimen included oral norfloxacin (400 mg twice per day), penicillin V (500 mg four times per day), fluconazole (200 mg per day), and valacyclovir (500 mg per day) or acyclovir. All were administered orally until hematopoietic recovery. Trimethoprin sulfamethoxazole was given orally for prevention of *Pneumocystis carinii* twice per day until day −2 and was restarted after hematopoietic recovery for 6 months. Broad-spectrum intravenous antibiotics were initiated for temperature greater then 38.3°C or for any sign of infection. Filgastrim 5 μg/kg/day was administered subcutaneously beginning 1 day after peripheral blood stem cell/ bone marrow infusion and was continued until engraftment. Blood products were transfused according to standard criteria.

Maintenance therapy included alpha interferon at a starting dose of 1×10^6 units administered subcutaneously three times per week and started when platelet count was $>50 \times 10^9$/l. In addition, dexamethasone 20 mg/m^2 was administered orally for 4 days each month. Ninety patients received maintenance therapy. Twenty-three were not treated due to early death or progression and seven for other reasons. Ten of these patients were treated with interferon alone and three with dexamethasone alone for various reasons. This maintenance regimen was usually continued until there was evidence of disease progression or patient intolerance.

Criteria for response

Response to induction therapy and to transplant was assessed by the criteria developed by the EBMT/ABMTR/IBMTR for evaluation of response.[27] Complete response (CR) was defined as the absence of monoclonal protein in serum and urine by both immunoelectrophoresis and immunofixation, a normal bone marrow and no increase in size or number of bone lesions. Partial response (PR) was defined as reduction of more than 50% in the level of serum monoclonal protein or $>$90% of urinary light chain excretion (or to less than 200 mg per day). All responses had to be maintained for at least 6 weeks. For patients with non-secretory disease responses were evaluated by serial bone marrow aspirations. CR required less than 5% plasma cells in the marrow and PR required 50% reduction of marrow plasmacytosis. No response was defined when the above criteria were not fulfilled. Relapse or progression required 25% increase in the level of paraprotein on two consecutive tests or reappearance of paraprotein after CR. For assessment of response in patients with refractory disease, post-transplant paraprotein levels were compared with the immediate pre-transplant level. When transplantation was performed as consolidation of prior response post-transplant paraprotein levels were compared with the level prior to the start of initial chemotherapy. The post-transplant response in patients with chemosensitive disease was by definition at least PR, unless they progressed or died within 6 weeks. Patients were evaluated for response at approximately 1, 2, 3, 6, 9 and 12 months after transplantation and at 6 month intervals thereafter.

Statistical analysis

The primary outcome variable for this study was overall survival (OS), defined from the day of autologous transplantation until death from any cause. Event-free survival (EFS) was defined as the time from the day of transplantation until either disease progression or death from any cause. Time to engraftment and treatment-related mortality were secondary end-points. Survival time distributions were estimated using the Kaplan–Meier method.[28] Distributions were compared between subsets of patients using the log-rank test. A tree-structured survival analysis was employed in order to construct a decision tree relating multiple pre-treatment patient characteristics to survival outcome.[29] The method is computer intensive and is a non-parametric exploratory tool since it does not depend on any

underlying distributional results. Continuous measures and categorical data were considered simultaneously; factors to be included were decided on the basis of a preliminary screening of individual factors. Initially, the program determines the optimal cutpoint of each possible predictor variable and then compares among variables to select the one which optimally splits the population into two subgroups most different in survival outcomes. The process is repeated in the resulting subgroups until no further partitioning is warranted. A sample re-use method termed cross-validation was used to confirm the final tree structure; this method deletes a random patient subset from the data, constructs a tree on the remaining data, and uses the group left out as new data on which to test the tree. As further confirmation of the multivariable results, a multiple regression analysis based on a proportional hazards model[30] was carried out with continuous-measure variables dichotomized either using cutpoints suggested by conventional use or by the tree analysis.

Results

Patient and disease characteristics

Between June 1991 and June 1998, 120 consecutive patients with multiple myeloma were included in the study. Patient and disease characteristics are shown in Table 1. Median age was 48 years (range 32–67). Fifty-two were Durie stage II and 68 stage III on presentation. The abnormal protein was IgG in 75 patients and IgA in 19. Median time from initial treatment to transplant was 330 days (range 88–3350) and patients received a median of 3 (range 1–5) prior treatments including the chemotherapy used for stem cell mobilization. Fifty-four patients were treated during first remission (43 patients) or chemosensitive relapse (11 patients). Forty-five patients were treated during primary refractory disease and 21 during chemo-refractory relapse. Patients with refractory disease had more prior treatments, longer interval from diagnosis, and a higher β2-microglobulin level. Patients with refractory relapse were more likely to receive bone marrow that was stored in prior remission rather than peripheral blood stem cells for cellular support.

Engraftment and toxicity

The median time to ANC $>0.5 \times 10^9$/l was 10 days (range 7–21) in those receiving peripheral blood stem cells *vs* 15 days (range 10–26) in those receiving bone marrow ($P < 0.001$). The median time to platelet count above 20×10^9/l was 11 days (range 6–67) and 29 days (range 12–46), respectively ($P = 0.002$). None of the other pre-transplant patient characteristics outlined in Table 1 predicted for a more rapid engraftment.

Sixteen patients (13%, 95% CI 8–21%) died of treatment-related causes. Six patients died of multi-organ failure with or without superimposed infection, one died of intracranial bleeding, nine patients died of infections including three patients who died of respiratory syncytial virus pneumonia. Univariate analysis indicated that treatment-related mortality (TRM) was associated with age above 55 years, with the use of bone marrow as stem cell source and with an earlier transplant year (before 1994; Table 2). Seven additional patients (6%) experienced non-fatal grade III

Table 1 Summary of patient characteristics by disease status at transplant

	Total	*Remission*	*Primary refractory*	*Refractory relapse*
No.	120	54	45	21
Age	48 (32–67)[a]	47 (32–67)	48 (39–65)	48 (34–59)
⩾55	18%	15%	20%	24%
Sex (% male)	64%	68%	58%	67%
Stage III	57%	53%	57%	67%
Heavy chain				
IgG	66%	55%	75%	71%
IgA	17%	20%	11%	19%
BJP[b]	9%	14%	5%	5%
IgD/NS[c]	9%	10%	9%	5%
Light chain λ	29%	36%	25%	18%
Prior tx ⩾3	50%	30%	62%	76%
Time to tx (days)	330 (88–3350)	275 (88–3350)	342 (88–2330)	520 (161–2920)
<1 year	58%	72%	53%	33%
Source of stem cell (% BM)[d]	20%	19%	9%	48%
Cyclosporine[e]	42%	43%	44%	33%
LDH[f]	480 (176–2023)	487 (176–1624)	425 (232–2023)	467 (202–1450)
>625	23%	24%	16%	37%
β2 microglobulin	3.0 (1.0–2.7)	2.3 (1.1–5.5)	3.5 (1.6–12.7)	3.5 (1.0–11.6)
⩾3 mg/dl	50%	23%	73%	58%
Creatinine	0.9 (0.5–1.8)	0.9 (0.5–1.8)	0.9 (0.5–1.7)	0.9 (0.5–1.5)

[a]Entries in table are median (range) unless otherwise noted.
[b]Bence Jones proteinuria only.
[c]IgD or non-secretory myeloma.
[d]Bone marrow as opposed to peripheral blood as the source of stem cells.
[e]Received cyclosporine for induction of autologous GVHD.
[f]Upper normal limit in the institution is 618 U/l.

Table 2 Treatment-related mortality by selected patient characteristics

Characteristic	*Total No.*	*Early deaths No. (%)*	*P value*
Total	120	16 (13)	
Age			
<55 years	98	9 (9)	0.02
⩾55 years	22	7 (32)	
Disease status			
Remission	54	4 (7)	
Primary refractory	45	7 (16)	0.06
Refractory relapse	21	5 (24)	
Source of stem cells			
Bone marrow	24	10 (42)	0.001
Peripheral blood	96	6 (6)	
Time to transplant			
<1 year	70	5 (8)	0.04
⩾1 year	50	11 (22)	
Year of transplant			
<1994	59	13 (22)	0.04
⩾1994	61	3 (5)	
β2 microglobulin			
<3 mg/dl	71	10 (14)	NS
⩾3 mg/dl	49	6 (12)	

toxicities by the Bearman criteria.[22] Three patients had hemorrhagic cystitis and required invasive treatment. This toxicity is unique to this regimen and related to high-dose cyclophosphamide. Two patients developed grade III GI toxicity, one patient hepatic toxicity and one lung toxicity. All grade III toxicities were reversible.

Response to high-dose chemotherapy

Of 54 patients with chemosensitive disease, 28 patients (52%) achieved CR including six patients in CR prior to transplant. Twenty-three patients (43%) remained in PR after the transplant with a median further reduction of myeloma protein of 70% (range 0–99%) in comparison with the pretransplant level. Three patients in this group (5%) were considered non-responders because of death or progression within 6 weeks of transplant.

The overall response rate of patients with primary refractory disease was 67% and that of patients with refractory relapse was 76%. The CR rate in these two groups of patients with refractory disease was 5%.

Survival rates after high-dose chemotherapy

The median follow-up of surviving patients was 29 months (range 7 months to 7.5 years). The estimated 5-year survival for the total group was 29% (95% CI 20–42%). The estimated 5-year EFS for the total group was 12% (95% CI 8–22%).

Survival was highest among patients achieving CR. These patients had a median survival of 59 months. Actuarial survival and progression-free survival were 56% and 32% at 5 years. Partial remission was associated with a median survival of 30 months, 25% of patients achieving a partial remission are alive and 10% progression-free at 5 years. The median survival of non-responders was only 11 months ($P = 0.001$).

Prognostic factors for survival

Table 3 summarizes the estimated survival by patient and disease characteristics measured prior to the start of high-dose chemotherapy. Patients for whom therapy was initiated while they were in first remission or responsive relapse had the longest survival, on average, while those with disease in refractory relapse had the shortest. Patients with primary refractory disease had survival that was intermediate between these groups. The estimated median survival of patients with chemosensitive disease was 48 months (95% CI 44 months to not available). The survival rates of patients in first remission and sensitive relapse were similar (data not shown). The estimated median survival was 35 months (95% CI 24–51 months) for patients with primary refractory disease and 9 months (95% CI 4–21 months) for patients with refractory relapse. The estimated median EFS was 23 months (95% CI 18–37 months), 10 months (95% CI 5–19 months), and 5 months (95% CI 3–9 months), respectively for each of these groups. The estimated 5 year survival is 46% for responsive disease, 26% for primary refractory, and 0% for refractory relapse. The estimates for 5 year EFS are 22%, 9% and 0%, respectively.

A tree-structured survival analysis was employed in order to consider the association of multiple patient characteristics with survival. With this approach it was possible to consider interaction among factors, to include continuous variables without specification of cutpoints, and to include factors which were unknown for some cases. Factors considered in the analysis were: age, stage of disease, serum

Table 3 Summary of survival by selected pretransplant characteristics

	Percentiles of survival time (months)			
	75%	*50%*	*25%*	*P value*
Total	9	38	80	
Disease status				
remission	30	48	NA	<0.01
primary refractory	7	35	60	
refractory relapse	3	9	20	
Stem cell source				
peripheral blood	20	38	NA	0.01
bone marrow	3	8	45	
Age				
<55	14	38	NA	0.03
⩾55	3	20	45	
LDH				
low	20	38	80	0.04
high	4	12	29	
Prior treatments				
<3	20	45	80	0.06
⩾3	6	27	54	
β2 microglobulin				
<3.0 mg/dl	11	44	80	0.06
⩾3.0 mg/dl	2	26	51	
Time to transplant				
<1 year	20	39	80	0.22
⩾1 year	4	27	60	
Cyclosporine				
treated	12	28	47	0.91
non-treated	8	38	NA	

protein type, disease status, interval from diagnosis to transplant, source of stem cells, pretransplant LDH and β2-microglobulin serum levels. The initial split in the tree was to divide patients into the three disease status categories. The only division within these subsets that could be cross-validated was the interval between diagnosis and transplant among patients with primary refractory disease (Figure 1). The tree analysis determined 134 days as the cutpoint. To be clinically relevant, we determined 6 months to be the cutpoint for improved survival. This added only three patients to the group and did not change the statistical significance. Although there were only 10 patients with primary refractory disease who were treated within 6 months of diagnosis they had superior survival (lasting 2+ to 7+ years). There was no step-wise shortening of survival for those transplanted beyond 6 months, suggesting that the advantage of early transplantation was limited to those patients with primary refractory disease transplanted within 6 months of starting treatment. Survival curves for the four groups of patients selected by the multivariable tree analysis are plotted in Figure 2. There was a wide separation among the survival curves. As the second approach to considering the association of multiple patient characteristics with survival, a proportional hazards model, was fitted using a stepwise procedure. The same potential factors were included in the analysis. Disease status was determined as the factor most associated with survival. The only additional factor for which there was evidence of association with survival after considering disease status was age. The tree-structured analysis did not identify any age cut-off as a prognostic factor. Age >55 years was used as the cut-off for the proportional hazards model and was found to be a significant adverse prognostic factor for survival by this model.

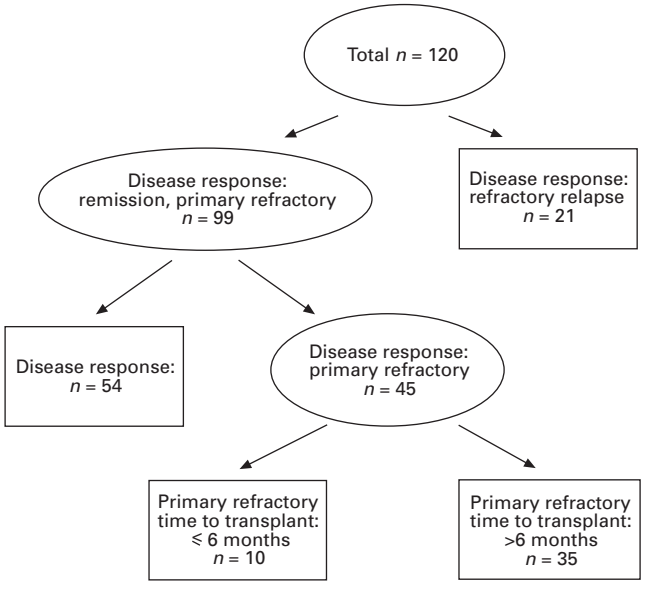

Figure 1 Tree-structured survival analysis of 120 patients with multiple myeloma after TBC and autologous hematopoietic transplantation. Four groups were identified: chemosensitive disease ($n = 54$), primary refractory transplanted within 6 months ($n = 10$) or later than 6 months ($n = 35$) from diagnosis, and patients in refractory relapse.

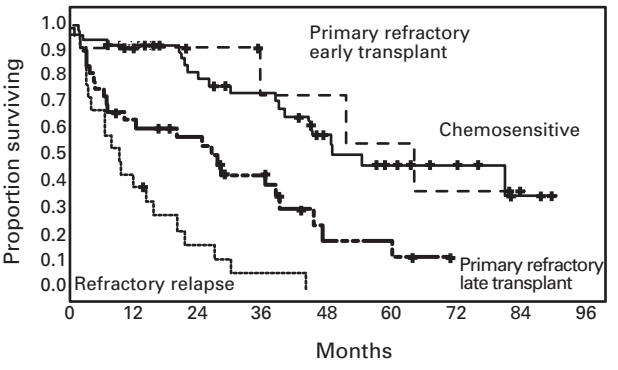

Figure 2 Kaplan–Meier curves of survival from the day of transplant by the risk groups determined by a tree-structured analysis. The four risk groups are as outlined in Figure 1.

Kinetics of response

Increasing response rates were observed over time after transplant while patients were on maintenance treatment alone. Twenty-four patients not at CR prior to transplant ultimately achieved CR. CR was achieved in a median of 146 days (range 15–536). Interestingly, four patients (17%) achieved CR between 6 and 12 months after transplant and two patients (8%) achieved CR more than 1 year after transplant while on maintenance treatment alone (Figure 3). Forty-six of the 66 patients with refractory disease, either primary or refractory relapse, achieved a response (PR or CR) after transplant (70%). Criteria for response were achieved in a median of 30 days (range 1–501). The median time to response was 56 days (range 1–501) for patients with primary refractory disease and 14 days (range 4–197) for refractory relapse. Forty patients achieved their response within 6 months of transplant (87%), and only three patients (6%) achieved their response after more than 12 months from the day of transplant.

The kinetics of response in patients with refractory dis-

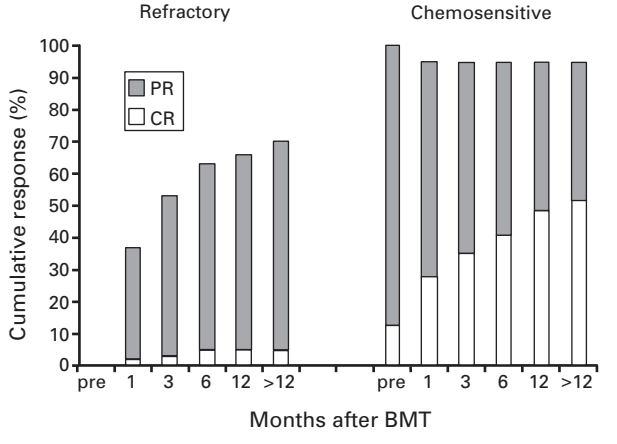

Figure 3 Cumulative response over time after hematopoietic transplantation. PR and CR rates are depicted in a cumulative fashion and show gradual increase in time. Responses are shown separately for patients with chemosensitive ($n = 54$) and patients with refractory disease ($n = 66$). Patients with chemosensitive disease were in at least PR after the transplant except for three patients who died or progressed within the first 6 weeks.

ease was found to correlate with survival. In Figure 4, survival subsequent to 1 month on treatment (landmark time) is plotted for those achieving response at 1 month and those still considered non-responders at that time. Estimated median subsequent survival for the 25 patients in the responding group was 6 months compared to 37 months for the 37 patients who had not responded at that time ($P < 0.001$). Twenty-one of the 36 patients who had not achieved a response by 1 month eventually went on to respond at a later time. Estimates of median subsequent progression-free survival were 20 months for the late responders *vs* 3 months for refractory patients achieving response criteria within a month of transplant. This correlation was also found when only patients with primary refractory were considered and even in a homogenous group of primary refractory patients transplanted within the first year (data not shown).

Discussion

High-dose chemotherapy and autologous hematopoietic transplantation has emerged as an effective treatment for patients with multiple myeloma. This treatment modality results in high response rates, however, almost all patients are destined to relapse. High-dose melphalan with or without total body irradiation has been the most frequently used conditioning regimen. We have investigated an alternative regimen trying to optimize cytoreduction prior to transplant in anticipation of better disease control if this goal is achieved. Dimopoulos *et al*[14] first reported on the combination of thiotepa, busulfan, and cyclophosphamide for the treatment of multiple myeloma. Each of the drugs used in this combination has proven activity against myeloma and has a different spectrum of extra-medullary toxicity, allowing escalation of the treatment doses and optimizing dose intensity.

We have treated 120 patients with the TBC combination. Overall, 81% of the patients responded and 26% achieving stringently defined CR. The median survival and EFS for the entire group was 38 months and 17 months, respectively. The multivariate analysis of pretransplant characteristics determined the disease status at transplant as the factor most highly associated with longer survival. Estimated median survival was 48 months for patients with chemosensitive disease and only 9 months for patients in refractory relapse. These results are comparable to other studies.[3–19] The outcomes in different studies are difficult to compare because of different patient populations and specifically different percentages of patients with refractory disease. Fifty-five percent of the patients in this study had refractory disease at the time of transplant. Harousseau *et al*[5] reported median survival of 54 months in patients with chemosensitive disease and 30 months in chemo-refractory disease with melphalan/TBI-based regimens. Bensinger *et al*[4] reported median survival of 36 months in a group of patients similar to our study and with a similar multi-alkylator regimen. Vesole *et al*[9] reported median survival of 41 months and a CR rate of 36% with the 'Total therapy' or tandem transplant program in previously treated patients, 38% with refractory disease. Longer median survival rates up to 57 to 68 months[10,11] have been reported in studies of newly diagnosed patients. However, these studies calculate survival from the day of diagnosis and include a lower percentage of patients with refractory disease. Although the outcomes seem comparable, comparison of outcomes following different regimens requires prospective comparative studies.

The only additional factor in this study for which there was evidence of association with survival after considering disease status was age. Other groups have not found age to be a prognostic variable.[11,31]

Unresponsiveness to conventional chemotherapy seems to have a different biological significance in patients early in the course of the disease. In the current study, 45 patients with primary refractory disease transplanted within 6 months of initiation of therapy had survival rates that were similar to those experienced by patients with chemosensitive disease despite lower CR rate. In contrast, patients with refractory relapse had extremely poor outcome with a median survival of less than 1 year despite a high overall response rate. Thus despite intensive therapy and a high remission rate, early disease recurrence and short survival indicated a need for other investigational approaches in this subgroup.

Early mortality for all patients in our series was 13%. This is similar to the report of Barlogie *et al*[6] for patients with advanced myeloma and during the same years. However, more recently with the introduction of peripheral blood stem cell transplants and better supportive care, the early mortality with this regimen was 4.8% (95% CI 1–13%). The toxicity profile was similar to that reported in other studies with the exception of severe hemorrhagic cystitis that occurred in 3% of all patients. The group most likely to benefit from TBC and autologous transplant were younger patients with chemosensitive disease. TRM was higher among older patients and patients with refractory disease. Caution should be taken when administering intensive regimens in these groups. These patients should be considered for alternative therapies.

The kinetics of paraprotein clearance after high-dose chemotherapy has not been well studied. Comparison of the response kinetics in different studies is difficult due to

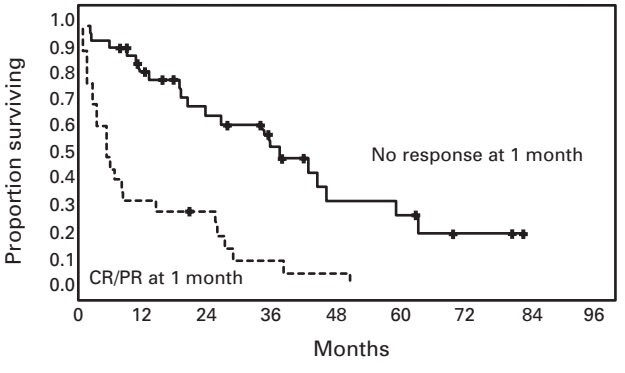

Figure 4 Kaplan–Meier curves of OS subsequent to 1 month after transplant (landmark analysis) for 62 patients with refractory disease at transplant, alive at that stage. Patients achieving response criteria at 1 month ($n = 25$) have a worse outcome than non-responders ($n = 37$) as determined at that time.

the use of different response criteria. Singhal *et al*[32] described the response kinetics in 33 patients in plateau phase with detectable protein. CR occurred in 96% of those ultimately achieving CR within 6 months with a median of 47 days. The time to CR was longer and the probability of achieving CR was lower with higher pretransplant paraprotein level. In a study of 50 previously untreated patients Gore *et al*[33] found that the median time to CR was 112 days (range 16–335) with seven of 25 patients achieving CR later than 6 months after transplant. Time to maximal response was 99 days in a similar series of previously untreated myeloma by Cunningham.[34] The prognostic significance of response kinetics in patients undergoing stem cell transplant for multiple myeloma remain unknown. In our series the median time to CR was 146 days (range 15–536). Six of 31 CRs were achieved after more than 6 months and two after more than 1 year from transplant. For patients with refractory disease, remission criteria were achieved in a median of 30 days (range 1–501); 12% of all responses were reached more than 6 months, and 6% more than 1 year after transplant. Maintenance therapy could have contributed to continuous and delayed responses. The study was not designed to assess the role of maintenance therapy since most responding patients were given long-term maintenance, however, delayed responses have also been noted in its absence.

Interestingly, refractory patients that achieved response criteria early after transplant had a worse outcome. Landmark analysis showed that survival subsequent to 1 month after transplant was superior in those not achieving remission by that time. The delayed clearance of paraprotein after high-dose chemotherapy, sometimes for more than 1 year, cannot be explained only on the basis of long half-life. The continuous slow reduction of paraprotein level suggests that intensive treatment may have a more pronounced effect on an early precursor or colony-forming cell. The more mature protein secreting cells gradually disappear when there is no ongoing self-renewal. It has been shown[35] that patients with refractory relapse have higher growth fractions and greater number of colony-forming cells in *in vitro* cultures. This explains the high response rate and rapid response but also rapid tumor regrowth and poor outcome. However, if the fraction of this more aggressive sub-clone is low, the response will be slow but durable. The pattern of response may be a clinical surrogate for the capability of the tumor for self-renewal and emergence of drug resistance and may have a significant prognostic value. Thus, patients with refractory disease who responded rapidly (within 1 month of transplant) probably had a more aggressive tumor with a high-dividing fraction, and despite a good initial response rapidly recurred with a resistant and rapidly fatal course. The same has been suggested by Hansen *et al*[36] for rapid response to conventional chemotherapy. Boccadoro *et al*[21] reported the same phenomenon in early responders to conventional chemotherapy when associated with high-labeling index.

The normal kinetics of response should be considered when evaluating post-high-dose chemotherapy treatments such as tandem transplants. The occurrence of CR after 6 months from high-dose chemotherapy suggests that at least part of the response to any post-transplant intervention, such as early administered second transplant, can still be related to the previous treatment. TBC might be an effective intensive regimen, given with a single transplant. With the improvement of supportive care it is as safe as the other regimens for autologous transplantation. TBC merits further study and comparison with alternative conditioning regimens as initial remission consolidation or early intensification of primary refractory disease.

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