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Myeloma

Fludarabine/melphalan conditioning for allogeneic transplantation in patients with multiple myeloma

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Summary:

The purpose of the study was to determine the feasibility and efficacy of a reduced intensity conditioning regimen of fludarabine and melphalan for allogeneic transplantation in patients with multiple myeloma. From August 1996 to December 2000, 22 patients received a reduced intensity conditioning regimen with fludarabine and melphalan. Median age was 51 years (range, 45–64), median time from initial therapy to transplant was 36 months (range, 3–135 months). Disease phase prior to transplant was primary refractory in two patients, refractory relapse in 11 patients, sensitive relapse in eight patients and initial remission consolidation in one patient. The median number of prior therapies was five (range, 1–7), and median beta 2 microglobulin prior to transplant was 3.0 mg/l (range, 1.0–7.3). All patients received unmanipulated grafts from either HLA matched sibling donors (n = 13) or matched unrelated donors (n = 9). Eighteen patients received fludarabine 30 mg/m^2 for 4 days with melphalan 140 mg/m^2 as a single dose and four patients received fludarabine 25 mg/m^2 for 5 days with melphalan 90 mg/m^2 daily for 2 days. All 21 patients evaluable for engraftment achieved a neutrophil count of $>0.5 \times 10^9$/l after a median of 12 days (range, 9–24), 18 patients achieved platelet transfusion independence after a median of 14 days (range, 8–47). All engrafting patients had 100% donor cell engraftment. Seven patients achieved a complete remission. Six patients are currently alive with a median follow-up of 15 months (range, 10–47 months). The actuarial survival and progression-free survival is 30 ± 11% and 19 ± 9% at 2 years. Non-relapse mortality at 100 days was 19 ± 10% and 40 ± 10% at 1 year. Fludarabine/melphalan combinations are feasible and allow consistent engraftment of allogeneic progenitor cells from both related and unrelated donors in patients with multiple myeloma and should be explored in patients with less advanced disease.

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Multiple myeloma is a neoplastic proliferation of plasma cells. Traditional approaches to therapy for myeloma have included systemic chemotherapy and radiation to affected sites.[1] Approximately 50% of patients will respond to initial therapy with alkylator-steroid combinations. Complete remissions occur in approximately 10% of patients and recurrence of disease is usually inevitable with emergence of resistant clones. Therefore, less than 15% of patients with myeloma will survive more than 10 years when treated with conventional chemotherapy alone.[1–3]

The use of high-dose chemo-radiotherapy with or without stem cell support was pioneered by McElwain and Barlogie.[4–6] High-dose chemotherapy resulted in a higher incidence of complete remission, and improved survival and progression-free survival when compared to conventional chemotherapy in both randomized and non-randomized trials.[7–12] However, recurrence of disease usually develops, and only a minority of patients remain progression-free 10 years after transplant.[13–15]

High-dose chemotherapy with allogeneic transplantation has the potential of adding a graft-versus-myeloma effect in addition to a stem cell product free of tumor cell contamination.[16,17] However, despite high complete remission rates of 30–50%, disease-free survival at 5 years after allografting is only 25%, due primarily to the high treatment-related mortality of 40 to 50%.[18–22]

Purine analog containing non-myeloablative chemotherapy can allow engraftment of allogeneic hematopoietic progenitor cells with acceptable toxicity in patients considered ineligible for standard myeloablative therapy and allogeneic transplantation either because of age or medical condition.[23–26] Therefore use of less intensive preparative regimens might provide an avenue for exploiting the graft-versus-myeloma effect without the toxicities seen with myeloablative therapies. We report the long-term results of our intial experience with a reduced intensity conditioning

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regimen of fludarabine and melphalan for patients with multiple myeloma undergoing allogeneic progenitor cell transplantion.[26]

Patients and methods

Eligibility criteria

Patients were eligible for this program if they had multiple myeloma and were considered poor candidates for autologous transplantation either because of disease status or inability to collect autologous stem cells. Patients up to the age of 65 years with a related HLA-compatible donor or fully matched unrelated donor were considered eligible if their Zubrod performance status was $\leqslant 2$, left ventricular ejection fraction was $\geqslant 40\%$, serum creatinine was $\leqslant 2.0$ mg/dl, bilirubin $\leqslant 3$ times upper limit of normal and transaminases $\leqslant 4$ times upper limit of normal. Both patient and donor signed written informed consent and were treated on protocols approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center. Unrelated donors were procured and consented through the National Marrow Donor Program according to currently accepted standards and procedures.

Treatment plan

From August 1996 to July 2000, all recipients of sibling donor transplants received fludarabine 30 mg/m^2 intravenously daily over 30 min on days -4, -3, -2, -1 and melphalan 140 mg/m^2 intravenously on day -1. Recipients of unrelated donor bone marrow were prepared with fludarabine 25 mg/m^2 intravenously daily for 5 days and with melphalan 90 mg/m^2 intravenously daily for 2 days until December 1998, after which they were conditioned with the same preparative regimens used for the sibling transplants. Donor-derived, filgrastim-stimulated peripheral blood stem cells or steady state bone marrow was infused on day 0. Drugs were dosed according to adjusted body weight in patients whose actual weight was 20% over ideal body weight.

Graft-versus-host disease (GVHD) prophylaxis consisted primarily of tacrolimus (FK506, Prograf, Fujisawa, Japan) given from day -2 until at least 3 to 6 months after transplant, in combination with methotrexate 5 mg/m^2 on days 1, 3, 6 and 11. Whole blood tacrolimus levels were monitored three times a week until day 90 post transplant, and weekly thereafter. Tacrolimus dose was modified to keep whole blood tacrolimus levels between 4 and 10 ng/ml. Antibacterial, antifungal and antiviral prophylaxis followed institutional protocols. This included trimethoprim/sulfamethaxozole for *Pneumocystis carinii* prophylaxis, acyclovir for herpes simplex virus prophylaxis, fluconazole for fungal prophylaxis, and penicillin and norfloxacillin or ciprofloxacillin for bacterial prophylaxis. CMV prophylaxis consisted of biweekly surveillance blood and urine cultures for CMV using shell vial and rapid antigen techniques. Recipients of unrelated or mismatched related donor transplants or patients on high-dose steroids received prophylactic ganciclovir 5 mg/kg intravenously five times weekly until day 100. Patients with CMV antigenemia or with positive shell vial cultures were treated with ganciclovir 5 mg/kg intravenously twice daily for 14 days, then daily for 8 weeks.

All patients received G-CSF 5 μg/kg subcutaneously daily from day +7 until achievement of an absolute neutrophil count (ANC) of $>1.5 \times 10^9$/l for 3 days in a row. Packed red blood cells were transfused to maintain hemoglobin levels $\geqslant 8$ gm/dl, and platelets were transfused to keep the platelet count $\geqslant 20 \times 10^9$/l. All blood products were filtered and irradiated to 1500 cGy.

Patients with neutropenic fever were treated with broad spectrum antibiotics according to institutional protocols. Patients developing grade 2 or greater acute GVHD received methylprednisolone at least 0.5 mg/kg every 6 h and could be enrolled on any existing protocol for the treatment of GVHD. Bone marrow aspiration along with serum and urine protein electrophoresis were carried out routinely 3–4 weeks after infusion and 3 and 12 months later and bone marrow underwent morphologic evaluation, FISH, cytogenetics and chimerism evaluation.

Statistical endpoints

Major endpoints included: neutrophil recovery defined as an ANC of $>0.5 \times 10^9$/l for 3 days in a row, and platelet recovery as a platelet count of $>20.0 \times 10^9$/l independent of platelet transfusions. Complete remission was determined by the European Bone Marrow Transplant Registry (EBMT)/International Bone Marrow Transplant Registry (IBMTR). Their criteria require the absence of a monoclonal protein on two consecutive immune fixation tests on blood and urine with a normal bone marrow examination in patients who had serum or urine monoclonal peaks. The criteria for complete remission are less stringent in patients with non-secretory myeloma who require only normal bone marrow examination with disappearance of extramedullary plasmacytomas.[27] Acute and chronic GVHD were scored according to published guidelines.[28,29] Chimerism was determined on bone marrow samples by cytogenetics or restriction fragment length polymorphisms using established techniques.[30,31] Toxicity was graded according to Bearman criteria.[32] Survival and disease-free survival calculations were analyzed as of 1 March 2001, using the techniques described by Kaplan and Meier.[33] Univariate analysis was done using the log–rank technique as determined by a commercially available statistical package (Statistica 2000, StatSoft Inc, Tulsa, OK, USA).

Patient characteristics

Patient characteristics are summarized in Table 1. The median age was 51 years (range, 46–64) and 11 patients had advanced refractory myeloma and nine had failed prior autologous transplant. The median number of prior therapies was five, and the median time to transplant from diagnosis was 36 months (range, 3–135 months).

Table 1 Characteristics of patients with multiple myeloma undergoing allogeneic transplants with a reduced intensity conditioning regimen of fludarabine melphalan at MDACC

n	*n* = 22
Age	51 (45–64)
Median time to transplant (months)	36 (3–135)
No. prior therapies	5 (2–9)
Auto-transplant failures	9
Disease status at transplant	
First remission	1
Primary refractory	2
Sensitive relapse	8
Refractory relapse	11
Immuneglobulin type	
Ig-G	12
Ig-A	5
Ig-D	1
Light chain only	3
Non-secretory	1
Pre-transplant β-2-microglobulin	3.0 (1.0–7.3)
Conditioning regimen	
FM140 mg/m^2	18
FM180 mg/m^2	4
Donor type	
6/6 Sibling	12
6/6 MUD	9
5/6 Sibling	1

n = number; FM = fludarabine/melphalan; MUD = matched-unrelated donor.

Results

Toxicity

The preparative regimen was well tolerated with only one instance of grade 4 toxicity in a patient who died with multiorgan failure and tumor lysis. Bearman toxicities ⩾grade 2 are summarized in Table 2. Four patients died before day 100, one from multiorgan failure and tumor lysis, and three from acute GVHD and infections. The 100-day non-relapse mortality rate was 19 ± 10%. Five additional patients died between day 100 and day 360 from non-relapse causes (GVHD with infections); thus, the 1 year non-relapse mortality rate was 40 ± 10%. Univariate analysis of pretransplant and transplant variables predicting for 100 day and 1 year non-relapse mortality is presented in Table 3. There was a trend for patients transplanted within 3 years of diagnosis to have a lower risk of non-relapse mortality at 1 year post transplant as compared to patients undergoing allografts greater than 3 years from diagnosis (23% *vs* 55%)

Table 2 Toxicities ⩾ grade 2 according to bearman scale

	Mucositis	*Liver*	*Heart*	*Kidney*	*Lung*	*Diarrhea*
Grade 2	0	2	1	4	0	6
Grade 3	0	0	0	0	0	0
Grade 4	0	1	1	1	1	0

Table 3 Univariate analysis of risk factors predicting for day 100 and day 360 NRM

Variable	*%NRM at day 100/day 360*	*P (Cox's F test)*
Age		
<50 (*n* = 9)	22/44	NS
>50 (*n* = 13)	16/38	
Patient sex		
M (*n* = 11)	9/34	NS
F (*n* = 11)	27/45	
Donor sex		
M (*n* = 16)	17/50	NS
F (*n* = 6)	19/38	
Time Dx to transplant		
<3 years (*n* = 11)	9/23	0.11
>3 years (*n* = 11)	27/55	
Disease stage		
Refractory relapse (*n* = 11)	27/46	NS
Other (*n* = 11)	9/36	
Donor type		
Sibling (*n* = 12)	17/42	NS
Mismatched or unrelated (*n* = 10)	20/40	

Engraftment and chimerism

Twenty-one patients achieved neutrophil recovery at a median of 12 days post transplant (range, 9–24). Eighteen patients achieved platelet transfusion independence at a median of 14 days post transplant (range, 8–47). Seventeen patients had chimerism analysis on day 30 of unsorted bone marrow cells with all patients having greater than 90% donor cell engraftment. No instances of secondary autologous reconstitution were observed, and at 1 year all six patients tested had 100% donor cells in unsorted bone marrow as determined by cytogenetics or molecular techniques.

Response: Sixteen patients (72%) responded according to the EBMT/IBMTR criteria. Seven patients (32%) achieved a complete remission, and nine achieved a partial remission (40%). Three patients who achieved CR progressed at 8, 17 and 19 months post transplant. Individual responses as well as subsequent responses to donor lymphocyte infusion are summarized in Table 4.

Graft-versus-host disease: Ten patients developed acute GVHD ⩾ grade 2 for an actuarial rate of 46% ± 10%. Six patients developed acute GVHD ⩾grade 3, for an actuarial risk of 27% ± 9%. No significant differences were observed among recipients of unrelated or mismatched transplants with regard to the risk of acute GVHD when compared to recipients of matched related donor transplants. Six patients developed chronic GVHD, all of these patients had extensive chronic GVHD.

Survival and progression-free survival: The overall survival for all patients is 30 ± 11% at 2 years with six patients currently alive at a median follow-up of 15 months (range, 9–47 months); the median survival for all patients was approximately 10 months. The progression-free survival for all patients was 19 ± 10% at 2 years with two

Table 4 Response to therapy and to donor lymphocyte infusions

UPN	*Sex/Age*	*Ig subtype*	*Months to transplant*	*Disease status at transplant*	*Donor type*	*Response*	*DLI*	*Survival (months)*	*Outcome*
930104	M/49	IgA	81.5	Refractory	MUD	PR	No	4.5	Died in PR with chronic GVHD
000093	M/45	BJP	49.6	Sensitive	Sibling	PR	Yes	10.3	Alive in PR, 8 weeks post DLI no GVHD
950300	M/47	IgG	58.5	Refractory	Sibling	SD	No	1.9	Died with GVHD
970013	F/54	IgG	36.0	Sensitive	Sibling	PR	Yes with Chemo	25.6	Died from disease. No response to DLI × 3.
970039	F/50	IgA	30.5	Sensitive	Sibling	CR	Yes with Chemo	29.4	Relapsed from CR. Responded to chemo and DLI. Relapsed and died from disease.
970071	F/53	IgG	16.9	Primary	Sibling	PR	Yes	47.2	Alive in PR, no definite response to DLI
930286	M/46	IgG	52.7	Refractory	Sibling	CR	No	5.8	Died chronic GVHD
960379	F/49	IgG	14.4	Refractory	Sibling	ED	No	0.16	Died multiorgan failure
970217	F/50	IgA	53.3	Sensitive	Sibling 1 Ag MM	SD	No	2.4	Died acute GVHD
980070	M/51	IgG	33.8	Sensitive	Sibling	CR	No	3.7	Died chronic GVHD and infection
980103	F/49	IgD	3.3	First rem	Sibling	CR	No	35.0	Alive in CR
980390	F/48	IgA	5.9	Refractory	Sibling	NR	Yes with Chemo	16.9	Died disease progression. No response to DLI
980408	F/50	IgA	16.5	Sensitive	Sibling	PR	No	4.4	Died chronic GVHD
980015	M/51	IgA	19.8	Refractory	MUD	CR	No	20.3	Alive in CR
990260	M/60	BJP	36.8	Refractory	Sibling	PR	Yes	8.4	Died disease progression no response to DLI
980194	F/53	IgG	33.9	Refractory	MUD	PR	Yes	9.6	Died disease progression and lung cancer. PR to DLI and chemotherapy
980397	M/64	IgG	53.6	Refractory	MUD	PR	No	10.2	Alive in PR
000221	F/57	BJP	109.4	Refractory	MUD	NR	No	2.2	Died acute GVHD
970143	M/55	IgG	13.3	Refractory	MUD	NR	Yes	10.4	Died disease progression. Transient PR to DLI
970204	M/49	IgG	41.8	Sensitive	MUD	CR	Yes	9.7	Died disease progression. No response to DLI
950192	M/48	NS	135.2	Refractory	MUD	CR	Yes	24.4	Died accident, 2 weeks post DLI for disease progression
910097	F/49	IgG	96.4	Sensitive	MUD	PR	No	5.1	Died chronic GVHD and infection

patients alive in continuing complete remission at 20+ and 35+ months, two patients with unmaintained partial remissions at 10 months, and one patient alive without progression after donor lymphocyte infusions, chemotherapy and thalidomide at 47 months. Univariate analysis on factors predicting for survival and progression-free survival have been summarized in Table 5.

Table 5 Univariate analysis on prognostic factors for progression free survival and overall survival in patients undergoing allografting after a fludarabine/melphalan conditioning regimen

Variable	*%OS @ 2 years*	*P*	*%PFS @ 2 years*	*P*
Age				
<50 (n = 9)	28	NS	17	NS
>50 (n = 13)	44		20	
Patient sex				
M (n = 11)	31	NS	12	NS
F (n = 11)	44		18	
Donor sex				
M (n = 16)	28	NS	19	NS
F (n = 6)	50		17	
Time Dx to transplant				
<3 years (n = 11)	41	0.13	27	NS
>3 year (n = 11)	33		0	
Disease stage				
Refractory relapse (n = 11)	32	NS	9	NS
Other (n = 11)	43		24	
Donor type				
Sibling (n = 12)	39	NS	22	NS
Mismatched or unrelated (n = 10)	36		10	

Discussion

The existence of a graft-versus-myeloma effect has been directly demonstrated by the responses seen to donor lymphocyte infusion in patients relapsing after conventional allogeneic stem cell transplantation.[16,17,34,35] Notwithstanding, it has not been possible to effectively exploit the graft-versus-myeloma effect of allogeneic transplantation due to the high rates of treatment-related deaths that have been observed in this patient population with this procedure. In previous comparative analyses, overall survival and progression-free survival were inferior in allografted recipients when compared to autografted patients despite a significantly lower relapse rate.[20]

Various investigators have postulated that by using reduced intensity conditioning regimens it would be possible to transplant older and debilitated patients, with less toxicity. The rationale for this approach is that less tissue destruction may result in a lower release of inflammatory cytokines and therefore a lower incidence of GVHD and other causes of non-relapse deaths.[23–25,36,37]

The reasons for exploring the combination of fludarabine and melphalan as a conditioning regimen for allogeneic transplantation in myeloma are derived from the following observations. First, melphalan is the most commonly used alkylating agent in myeloma, it has well documented activity against resistant myeloma at high doses and is well tolerated with little extramedullary toxicity even in older and debilitated patients.[38,39] Second, fludarabine is a potent immunosuppressive agent that can inhibit the mixed lymphocyte reaction *in vitro* and facilitate engraftment.[39] Third,

purine analogs can inhibit the mechanisms of DNA repair that come into effect after alkylator-induced damage, thus potentially enhancing the antimyeloma effect of melphalan.[39,40]

Fludarabine-melphalan combinations used as described here resulted in universal donor cell engraftment with no instances of primary or secondary autologous reconstitution. Lower doses of melphalan (100 mg/m^2) without fludarabine, have been showed by Badros *et al*[41] to result in high rates of engraftment, except for one instance of secondary autologous reconstitution and three instances of mixed chimerism. These results contrast with the results of this study in which no instances of mixed chimerism or autologous reconstitution were seen. That difference suggests a role for fludarabine in facilitating engraftment of allogeneic stem cells, and that doses of melphalan below 100 mg/m^2 might be associated with higher rates of graft failure.

The immediate toxicity of the regimen was mild, but the 40% incidence of non-relapse deaths at 1 year remains excessive. The data in this report suggest that better patient selection could reduce non-relapse deaths, since patients transplanted within 3 years of diagnosis had a 1-year non-relapse mortality rate of 23%. GVHD was the second most common cause of death aside from disease progression. Future strategies to prevent GVHD in the setting of nonablative transplantation include addition of a monoclonal antibody against CD52 (CAMPATH 1-H) and addition of mycophenolate-mofetil.[42,43] Peggs *et al*[42] recently reported that the use of pretransplant CAMPATH 1H was associated with a lower incidence of moderate and severe acute GVHD; however, it is unknown whether the reduction in rates of GVHD may translate into a higher relapse rate, and inferior outcomes because of disease progression.[42]

The primary cause of treatment failure was disease recurrence which caused the death of eight patients. Thus, despite achieving donor cell engraftment, the graft-versus-myeloma effect did not result in long-term disease control. In this report, 10 patients received DLIs either because of relapse or failure to achieve complete remission after withdrawal of immunosuppression. Only three of these patients responded to DLI or withdrawal of immunosuppression. Of interest, most patients in this study were treated for chemorefractory relapses; in a recent update by Lokhorst *et al*[17] chemosensitivity was an important predictor for response to donor lymphocyte infusions in myeloma. Therefore, to optimize the results of non-ablative and reduced intensity conditioning in myeloma it will be necessary to treat patients earlier in the course of their disease when the disease is more sensitive to therapeutic maneuvers.

This initial experience with reduced intensity conditioning for multiple myeloma demonstrates that durable donor cell engraftment can be achieved, and a small fraction of patients with advanced resistant disease can achieve long-term disease control. This and other reports seem to confirm that non-relapse mortality rates are lower than with conventional ablative therapy. Among patients transplanted less than 3 years from diagnosis, the non-relapse mortality rate at 100 days was 9%, and at 1 year 23%. Similarly, the EBMTR reported a non-relapse mortality rate at 1 year of 10% in patients with myeloma who were allografted early during the course of their disease.[44] These observations underscore the fact that optimal use of allogeneic transplantation depends on appropriate timing of the procedure.[20] The optimal non-ablative transplant strategy is unknown. Molina *et al*[43] reported that a regimen of low-dose total body irradiation (200 cGy) was associated with a high risk of graft failure in patients with myeloma who had never received intensive therapy. However, when this preparative regimen is given within 3–9 months of high-dose melphalan, the use of low-dose TBI was associated with universal donor cell engraftment, a high response rate and a low non-relapse mortality. The advantages of different reduced intensity strategies will need to be determined by carefully designed trials.

This analysis was not intended to define the importance of the stem cell product in determining transplant outcomes. All recipients of unrelated donor progenitor cells received bone marrow, while all other patients received filgrastim stimulated bone marrow. Most peripheral blood stem cell recipients received within 4–5 $\times$ 10^6 CD34$^+$ cells/kg. Therefore with the small number of patients and the homogeneity of stem cell dose, meaningful differences would be difficult to detect and the analysis was not performed. However, further study of non-ablative transplantation in a more homogeneous group of newly diagnosed myeloma patients or as first salvage therapy will need to look at cell doses and cell source as important variables for outcome.

Non-ablative and reduced intensity conditioned allografts as frontline therapy for multiple myeloma should still be confined to clinical trials. With the current results of autografting and thalidomide, autologous transplantation should still be considered the frontline treatment of choice for most patients with multiple myeloma. However, patients with chromosome 13 abnormalities, and those with high tumor masses refractory to induction therapy could be considered for frontline allografting if an HLA-compatible donor is available, since results of autologous transplantation in these settings have been poor.[11,45,46] Notwithstanding the limited experience to date, it is possible to say that further exploration of allogeneic transplantation for myeloma should only be undertaken in the context of non-ablative and reduced intensity conditioning regimens, with little if any role for conventional myeloablative therapies.

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