

Improved Outcome of Allogeneic Transplantation in High-Risk Multiple Myeloma Patients After Nonmyeloablative Conditioning

By Ashraf Badros, Bart Barlogie, Eric Siegel, Michele Cottler-Fox, Maurizio Zangari, Athanasios Fassas, Christopher Morris, Elias Anaissie, Frits Van Rhee, and Guido Tricot

Purpose: We present our experience with relapsed and recently diagnosed patients with high-risk multiple myeloma (MM) receiving immunosuppressive, nonmyeloablative melphalan (MEL)-based conditioning regimens (mini-allograft).

Patients and Methods: Thirty-one MM patients received allografts from HLA-matched siblings (n = 25) or unrelated donors (n = 6) using a mini-allograft. Seventeen had progressive disease (PD) and 14 had responsive disease (RD) (six with primary RD and eight with responsive relapse). Thirty patients had received one (n = 13) or two or more (n = 17) prior autologous transplantations (ATs). Median age was 56 years (range, 38 to 69 years). Twenty-one patients had chromosome 13 abnormality. Two patients were hemodialysis dependent. Blood and bone marrow grafts were administered to 28 and three patients, respectively. Donor lymphocyte infusions were given to 18 patients either to attain full donor chimerism (n = 6) or to eradicate residual disease (n = 12).

Results: By day 100, 25 (89%) of 28 patients were full donor chimeras, one was a mixed chimera, and two

had autologous reconstitution. Acute graft-versus-host disease (GVHD) developed in 18 patients (58%), and 10 progressed to chronic GVHD (limited in six and extensive in four). At a median follow-up of 6 months, 19 (61%) of 31 patients achieved complete/near complete remission. Twelve patients (39%) have died: three of PD, three of early treatment-related mortality (TRM) (before day 100), and six of late TRM. Median overall survival (OS) was 15 months. At 1 year, there was a significantly longer event-free survival (86% v 31%, P = .01) and OS (86% v 48%, P = .04) when a mini-allograft was performed after one versus two or more prior ATs, respectively. When compared with historical MM controls (n = 93) receiving conventional allografts, early TRM was significantly lower (10% v 29%, P = .03), and OS at 1 year was better (71% v 45%; P = .08) in the mini-allograft MM patients.

Conclusion: Mini-allograft induced excellent disease control in MM patients with high-risk disease, but is still associated with a significant GVHD.

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MULTIPLE MYELOMA (MM) is a chemotherapy-refractory hematologic malignancy with a less than 5% complete remission (CR) rate, and less than 5% of the patients survive 10 years with conventional therapy.¹ Autologous transplantation (AT) induces CR rates in approximately 50% of newly diagnosed patients, which can be durable in the subset of patients with no chromosome 13 abnormalities and low beta 2-microglobulin (β_2 -microglobulin) at diagnosis.²⁻⁴ However, relapse continues to be a major complication after AT.

Allogeneic stem cell transplants are curative in 10% to 20% of the patients with various refractory hematologic malignancies and in a larger proportion of patients treated in remission.^{5,6} The role of allografting in MM patients, however, remains controversial.^{7,8} Treatment-related mortality (TRM) in the first 100 days is 30% to 50% in most published series.⁹⁻¹¹ However, 30% to 50% of patients who survive the first year remain disease-free at 3 to 6 years, with well-documented cases of sustained molecular remissions.^{12,13} Such durable remissions have been attributed to a graft-versus-myeloma (GVM) effect.¹⁴⁻¹⁶ Further support for a GVM effect comes from donor lymphocyte infusion (DLI)-induced remissions after allogeneic transplant relaps-

es.¹⁷ In a previous report we have shown that a nonmyeloablative dose of melphalan (MEL) 100 mg/m² followed by allogeneic stem cells leads to prompt engraftment and induces excellent remissions in MM patients with resistant disease.¹⁸

We now present our experience with relapsed and recently diagnosed patients with high-risk MM receiving immunosuppressive, nonmyeloablative MEL-based conditioning regimens (mini-allograft). We compare our recently treated mini-allografted patients with historical controls who received the "standard" conditioning regimen.

From the Myeloma and Transplantation Research Center, University of Arkansas for Medical Sciences, Little Rock, AR.

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Address reprint requests to Ashraf Badros, MD, University of Maryland, Greenebaum Cancer Center, 22 South Greene St, Baltimore, MD 21201; email: abadros@umm.edu.

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PATIENTS AND METHODS

Study Design

Patients and donors in the study provided written informed consent that was approved by the institutional review board of the University of Arkansas for Medical Sciences. The conditioning regimen for the recipients consisted of intravenous MEL 100 mg/m² over 20 minutes on day -1 for those receiving a graft from siblings, whereas recipients of unrelated donor grafts received the same dose of MEL plus 250 cGy of total-body irradiation in two fractions on day -2 and fludarabine 30 mg/m² on days -2 and -1. The outcome of the first 16 patients on the protocol has been reported previously.¹⁸

Cyclosporine was given intravenously (3 mg/kg/d) to all patients starting on day -1, with a target level of 300 ng/ μ L. Patients receiving unrelated donor grafts received in addition methylprednisolone 1 mg/kg/d starting on day 5 that was tapered and completely stopped by day 29 if there was no evidence of GVHD. No patient received methotrexate for graft-versus-host disease (GVHD) prophylaxis.

HLA-matched siblings were mobilized with granulocyte colony-stimulating factor (G-CSF) 10 μ g/kg/d. On day 4 of G-CSF, large-volume leukapheresis (> 15 L) was performed. Mobilized peripheral-blood stem cells were also collected on days 5, 6, and 7 if necessary to attain 10 \times 10⁶ CD34⁺ cells/kg of the patient's weight. Unrelated donors had a choice between receiving G-CSF followed by leukapheresis or undergoing bone marrow harvest. On day 0, unmanipulated stem cells were infused, except in two recipients of unrelated donor grafts, where T-cell depletion was performed using CD34⁺ cell selection (Isolex; Nexell Therapeutics, Inc, Irvine, CA). The graft target was a maximum stem cell infusion of 5 \times 10⁶ CD34⁺ cells/kg; the rest was stored for later use as DLI.

In the absence of GVHD, cyclosporine was tapered over 1 month starting on day 60 after mini-allograft. DLIs with escalating doses of donor CD3⁺ lymphocytes were scheduled on days 21, 42, and 112 to establish full chimeric engraftment in patients with no GVHD. Initially, patients received all scheduled DLIs. Later in the study, because of a high incidence of GVHD, cyclosporine was continued until day 120, and decisions regarding DLI were made on the basis of the chimeric and disease status of the patients. Those with mixed chimerism received additional donor cells while on cyclosporine in the absence of acute GVHD. Patients with residual or progressive disease had cyclosporine discontinued, and in the absence of GVHD, they received additional DLI with no GVHD prophylaxis. Patients who relapsed or had no response to DLI received salvage chemotherapy followed by additional DLI.

Antimicrobial prophylaxis consisted of acyclovir 400 mg every 8 hours from day 1 to 6 months after transplant, and itraconazole 400 mg/d until the CD4⁺ count exceeded 400/ μ L. Patients received levofloxacin 500 mg/d from day 0 to 2 years after transplantation and cotrimoxazole bid twice a week from the time of neutrophil recovery to 6 months. Patients at high risk of fungal infection and those unable to tolerate oral itraconazole received intravenous liposomal amphotericin B 1 mg/kg/d. Blood samples were checked each week for cytomegalovirus (CMV) antigenemia; those who tested positive were treated preemptively with ganciclovir. Patients received intravenous immunoglobulin 0.5 g/kg if the immunoglobulin G level was less than 500 mg/dL.

Study End Points

The end points of the study were engraftment, chimerism, toxicity, TRM, incidence of GVHD, and MM response. Chronic

GVHD was evaluated in patients with at least 3 months' follow-up. The outcome of the mini-allograft patients was compared with historical patients who received standard allotransplants at the University of Arkansas.

Engraftment was defined as neutrophil recovery to more than 0.5 \times 10⁹/L for 3 consecutive days and untransfused platelet count greater than 20 \times 10⁹/L. The degree of donor-recipient chimerism was assessed by polymerase chain reaction assay of short tandem repeat loci from blood and marrow according to published methods.¹⁹ Studies for chimerism were assessed monthly for 1 year. Calculating the percentage of donor DNA in comparison with the pretransplant donor and recipient genotype quantitated chimerism. The assay is able to detect chimerism if more than 5% donor or host DNA is present. Mixed chimerism was defined as the presence of more than 5% donor and host-derived cells on more than one occasion in the whole blood. Acute GVHD was assessed according to the criteria of the International Bone Marrow Transplant Registry. Other toxicities were assessed using National Cancer Institute criteria. Chronic GVHD was defined as GVHD occurring 100 days or more after bone-marrow transplant and was graded as none, limited, or extensive.

CR required the disappearance of monoclonal gammopathy in serum and urine on immunofixation analysis and normal bone marrow aspirate and biopsy (< 5% plasmacytosis) on two successive occasions 2 months apart. Near CR (nCR) was defined as a CR with only positive immunofixation by electrophoresis. Partial remission (PR) required a bone marrow with 5% to 10% plasmacytosis and a 75% reduction in M-protein. Early TRM included any death within 100 days after mini-allograft. Late TRM was defined as any death after day 100 attributed to complications of the allograft. Relapse was defined as recurrence of monoclonal protein or bone marrow plasmacytosis in case of CR. Progressive disease for non-CR patients implied at least a 25% increase in M-protein and/or bone marrow plasmacytosis or the development of new extramedullary disease.

Data are presented as of March 15, 2001. Statistical analyses were performed using SAS Version 8.0 (SAS Institute, Inc, Cary, NC). Kruskal-Wallis and Fisher's exact tests were applied to discern imbalances in patient characteristics in the mini-allograft versus standard allograft and outcome. The Cochran-Mantel-Haenszel test was used to detect trends in outcome according to number of prior ATs and disease status. The Bowker test of symmetry for matched-pair data was performed to determine whether patients' disease status showed net improvement after the allograft. The log-rank test was applied to assess differences between Kaplan-Meier survival curves.

Difference in overall survival (OS) between mini-allografts and standard allografts was adjusted for clinical covariates using multivariate Cox regression. Covariates considered for multivariate regression were age, albumin less than 3.5 g/dL, β_2 -microglobulin greater than 4 mg/L, creatinine greater than 2 mg/dL, C-reactive protein greater than 1 mg/dL, disease status at allografting, hemoglobin less than 10 g/dL, lactate dehydrogenase greater than 190 U/L, number of prior ATs, sex, time from diagnosis and from last AT, and cytogenetic abnormalities (including those involving chromosomes 11 and 13). Mini-allograft and standard allograft plus the above covariates were entered into the initial multivariate model for variable selection. The final multivariate model was restricted to significant covariates for OS in the initial model plus transplant mode (mini-allograft *v* standard allograft).

Table 1. Patient Characteristics at Mini-Allograft and Standard Allograft

	Mini-Allograft (n = 31)		Standard Allograft (n = 93)		P
	No.	%	No.	%	
Age, years					
Median		56		46	< .0001
Range		38-69		30-63	
Female	13	41	33	35	.50
IgA isotype	4	13	28	30	.06
Dialysis-dependent	2	6	0		.06
Abnormal chromosomes	30	97	58	62	.0003
Chromosome 13	21	68	33	35	.002
MDS	4	13	0		.003
No. of patients:					
< 2 prior ATs	14	45	73	78	.001
≥ 2 prior ATs	17	55	20	22	
Disease status at allograft					
Responsive	14	45	26	27*	.2
Progressive	17	55	60	65*	
Time from Dx to Tx, months					
Median		29		20	.04
Range		8-164		9.6-74	
Time from prior AT to Tx, months					
Median		12		8	.2
Range		2.7-66		2.4-54	

Abbreviations: IgA, immunoglobulin A; Tx, allograft; Dx, diagnosis.

*Excluding seven patients with inadequate data.

RESULTS

Patients

Patients' characteristics before mini-allografts are listed in Table 1. The population included 31 MM patients who were ineligible for conventional myeloablative conditioning because of prior AT, age, and/or comorbidity. The patients ranged in age from 38 to 69 years (median, 56 years). Median time from diagnosis to mini-allograft was 29 months (range, 8 to 164 months). All but one patient had received either one prior AT (13 patients), two ATs (14 patients), or three prior ATs (three patients). The median time from the last AT to mini-allograft was 12 months (range, 2.7 to 66 months). Twenty-five patients had been treated with salvage thalidomide after relapse. At the time of allografting, 17 patients had progressive disease (PD) and 14 patients had chemosensitive disease. Of the chemosensitive patients, six were primary responders (two were in CR, two were in nCR, and two were in PR), whereas eight had responsive disease after relapse, with three patients in PR, four in CR, and one in nCR. All patients had at least one poor prognostic factor as defined by the presence of high β_2 -microglobulin, complex cytogenetics, or relapse after prior AT. A complex cytogenetic profile was present in 30 patients; partial or complete deletion of chromosome 13 was present in 21. Cytogenetic and clinical evidence of myelo-

dysplastic syndromes (MDSs) in addition to active myeloma was observed in four patients. β_2 -Microglobulin before allograft was elevated (> 4 mg/L) in seven patients. Before mini-allograft, the median number of CD4⁺ lymphocytes was 209/ μ L (range, 35 to 630/ μ L).

Characteristics of the donors for the mini-allograft patients are summarized in Table 2. Of the 31 patients, 28 received a fully HLA-compatible graft as determined serologically for class I (A, B, and C loci) and molecularly for class II (DRB1 and DQB1 loci). Three patients received grafts mismatched at one class II HLA locus (two sibling

Table 2. Donor Characteristics of Mini-Allograft Patients

	Sibling (n = 25)	Unrelated (n = 6)
Sex mismatch	15	2
PBSCs/BM graft	25/0	3/3
HLA mismatch	2	1
ABO mismatch		
Major	5	2
Minor	3	0
CMV*		
Donor+ / patient+	12	4
patient+ / donor-	4	1

Abbreviations: PBSCs, peripheral-blood stem cells; BM, bone marrow.

*CMV status of patient and donor.

Table 3. Chimerism After Mini-Allografts

	Related (n = 25)	Unrelated (n = 6)
Conditioning regimen		
MEL	25	1
MEL/TBI/fludarabine	0	5
T-cell depletion	0	2
GVHD prophylaxis		
Cyclosporine alone	24	0
Cyclosporine/methylprednisolone	1	6
Day 30		
Full donor chimera	20	5
Mixed chimera	5	1
Day 100	n = 24*	n = 4*
Full donor chimeric	22	3
Mixed chimeric†	1	0
Autologous reconstitution	1	1

*Three full chimera patients at day 30 were not evaluable at day 100 because of death.

†Mixed chimerism indicates the presence of > 5% donor and host-derived hematopoiesis.

donors) or one class I HLA locus (one unrelated donor). Twenty-eight patients received G-CSF–mobilized grafts, and three received marrow from unrelated donors.

Engraftment and Chimerism

Patients received a median of 4.8×10^6 CD34⁺ cells/kg (range, 1.5 to 12.5) and 1.7×10^8 CD3⁺ cells/kg (range, 0.3 to 5.1). Two patients had received T-cell–depleted grafts containing 1×10^5 CD3⁺ cells/kg. G-CSF was not rou-

tinely administered after transplantation. Five patients received G-CSF by day 16 because of slow white blood cell count recovery. Neutrophil recovery greater than $500/\mu\text{L}$ was reached at a median of 14 days (range, 10 to 46 days); the median time to an unsupported platelet count greater than $20 \times 10^9/\text{L}$ was 15 days (range, 0 to 50+ days). Five patients did not require any platelet support; the median number of platelet transfusions was eight (range, zero to 70+). The median number of units of RBCs transfused was 5 (range, 2 to 15), 13 (range, 5 to 18), and 14 (range, 6 to 35) for patients with full ABO matched (n = 21), minor (n = 3), or major mismatched (n = 7) donors, respectively.

At 21 to 30 days after transplantation, 25 of 31 patients were full donor chimeras (Table 3). Two patients (including one who received an unrelated T-cell–depleted graft) failed to engraft even after an additional allogeneic stem cell infusion. Eventually, both patients received an autologous stem cell rescue; one achieved and is maintaining an nCR more than 1 year after the mini-allograft, whereas the other had progressive disease. Interestingly, four of seven patients with major ABO mismatches were mixed chimeras at day 30, including the two patients who rejected their allografts. By day 100, 25 (89%) of 28 assessable patients were full donor chimera.

Disease Response

The data on clinical response after mini-allograft are listed in Table 4. In these 31 poor-prognosis patients, 19 (61%) had an excellent response after mini-allograft, with a

Table 4. Outcome After Mini-Allograft

Variable	No.	AGVHD	CR/nCR	PR	PD	NA	Mortality
Age							
≤ 55 years	15	10	8	2	4	1	4
≥ 55 years	16	8	11	1	1	3	8
HLA							
Sib	25	15	16	3	4	2	10
MUD	6	3	3	0	1	2	2
No. of ATs							
0	1	0	1	0	0	0	0
1	13	8	11	0	1	1	4
2	14	7	5	3	4	2	6
3	3	3	2	0	0	1	2
							P = .06*
Status at allograft							
CR/nCR	9	5	8	0	0	1	1
PR	5	3	4	0	1	0	2
PD	17	10	7	3	4	3	9
							P = .04*
							P = .007†

Abbreviations: AGVHD, acute graft-versus-host disease; NA, not assessable; Sib, sibling; MUD, unrelated matched donor.

*Cochran-Mantel-Haenszel test for trend.

†Bowker test for symmetry.

Table 5. Transplant-Related Adverse Effects After Mini-Allotransplants

	Sibling (n = 25)		Unrelated (n = 6)	
	No. of Patients	Fatal Events	No. of Patients	Fatal Events
Overall mortality	10		2	
TRM (first 100 days)	1		2	
Progressive disease	5	3	0	
Acute GVHD > grade II	15		3	
Chronic GVHD	9/22		1/4	
Bacteremia*	10		2	
Sepsis†	3	2	0	
CMV antigenemia	14	1	2	
Aspergillosis	2	1	1	1
Toxoplasmosis	1	1	0	
Seizures	2		0	
Pneumonitis	6	2	2	1
Pure red-cell aplasia	0		1	

*Bacteremia is detection of an organism in the blood.

†Sepsis is bacteremia with systemic evidence of infection (eg, low blood pressure, decreased urine output).

stringently defined CR achieved in 12 patients and nCR in seven (only immunofixation-positive). Three other patients achieved a PR.

None of the four patients with MM and MDS before mini-allograft had cytogenetic or clinical evidence of MDS or MM after allografting. Three are alive and disease free (at 9+, 15+, and 36+ months), and one died of pneumonia on day 400 in CR.

Not unexpectedly, myeloma responses in refractory patients were only seen after the development of acute GVHD. Status at mini-allograft significantly affected the post-allograft responses: patients who underwent transplantation in CR/nCR remained in CR/nCR, and none had relapsed at last follow-up, four of five patients who received allograft in PR had achieved nCR/CR, and only 10 of 17 patients with PD before the allograft had a response (nCR/CR in seven patients and PR in three). Overall, 14 patients improved after allograft and one worsened ($P = .007$). A total of five patients had evidence of PD after mini-allograft; three of them had received two prior ATs and had nonresponsive disease before mini-allograft.

Donor Lymphocyte Infusions and Subsequent Therapy

As stable allografts were established after a single infusion of allogeneic stem cells with a high incidence of GVHD noted in the first phase of the study, the routine use of preemptive DLI was limited. DLIs were only given to establish full chimerism or to patients with evidence of extensive residual/or progressive disease. For the whole group, 18 patients received DLI: five patients received three DLIs, five patients received two DLIs, and eight patients received only one DLI. Six patients received DLIs to attain full donor chimerism and 12 to eradicate residual disease.

Three patients had a persistent large tumor burden. They received dexamethasone, cyclophosphamide, etoposide, and cisplatin chemotherapy before DLI on day 60. All three patients developed clinical grade 2/3 GVHD; two attained CR and one a PR.

Three patients with evidence of progressive disease after day 100 of the mini-allograft received salvage therapy with dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, and etoposide and DLI. One patient who had an extramedullary relapse with a large renal mass and CNS involvement achieved and sustained a CR, but eventually died of GVHD complications at day 514. One is in stable PR at day 400 and one died with PD at day 320. CR attained after salvage chemotherapy and DLI were not included in the overall responses (19 in CR/nCR and three in PR) reported after mini-allograft.

Toxicity and Mortality

In this cohort of heavily pretreated, high-risk MM patients, this mini-allograft protocol was reasonably tolerated in both related and unrelated settings in the early posttransplant phase. The major regimen-related toxicities observed are listed in Table 5.

Eighteen patients developed acute GVHD (> grade 2 in 16 and skin grade 1 in two), and 12 developed GVHD for the first time after donor lymphocyte infusions. Grade 2 GVHD responded well to methylprednisolone and cyclosporine. Ten patients developed chronic GVHD; six had extensive chronic GVHD. The incidence of GVHD was not affected by age ($P = .7$), the number of prior ATs (0.2), or the disease status at mini-allograft ($P = .9$) (Table 4).

Pneumonitis developed in eight patients, in association with engraftment in four; three of eight patients required

ventilator support. One patient developed bronchiolitis obliterans and eventually died of bacterial pneumonia on day 400. Veno-occlusive disease was not observed. Most patients had more than one episode of fever. Eight patients had coagulase-negative *Staphylococcus* bacteremia. There was a high incidence of CMV antigenemia in the first 16 patients. All were CMV antibody-positive before mini-allograft and had a CMV-positive donor. This complication was successfully treated with ganciclovir. No CMV disease developed in any of the patients. The last 15 patients received preconditioning ganciclovir, and only three patients had evidence of CMV activation during the first 100 days. Invasive aspergillosis developed in three patients after steroid therapy for GVHD. One patient had seizures secondary to high cyclosporine level. Pure red cell aplasia developed in one patient who received bone marrow from an unrelated donor with major ABO mismatch.

Overall, 12 patients have died. TRM in the first 100 days was 10% ($n = 3$). All were very-high-risk patients (one was hemodialysis dependent, and one had received three prior ATs, with a history of diffuse interstitial pneumonitis); two had received unrelated donor grafts. Three patients died of PD and six including five patients in CR/nCR died from late complications including sepsis ($n = 3$), aspergillosis ($n = 2$), and toxoplasmosis ($n = 1$), which were secondary to immunosuppressive therapy for GVHD. Disease status at the time of mini-allograft significantly affected overall mortality: there was one death (11%) among nine patients who underwent transplantation in CR/nCR, versus two (40%) of five patients who underwent allografting in PR and nine (64%) of 14 patients who underwent allografting with PD ($P = .04$) (Table 4). There was a trend for higher mortality for patients who received two or more ATs before mini-allograft ($P = .06$) (Table 4).

Survival

With a median follow-up of 6 months (range, 45 to 730 days) 19 patients are alive with Karnofsky performance status scores of 90 to 100. The median overall durations of event-free survival (EFS) and OS were 15 months, and the projected actuarial survival at 1 and 2 years is 71% and 31%, respectively (Fig 1). The number of prior ATs significantly affected EFS and OS (Fig 2). At 1 year, there was a significantly longer EFS (86% v 31%, $P = .01$) and OS (86% v 48%, $P = .04$) when mini-allograft was performed after one versus two or more prior ATs. In a subset analysis and with a short follow-up, patients with responsive disease (CR, nCR, and stable PR) who underwent mini-allograft after one prior AT ($n = 9$) had a remarkable 100% EFS and OS. The difference between this subset and all the other patients (PD and/or \geq two prior

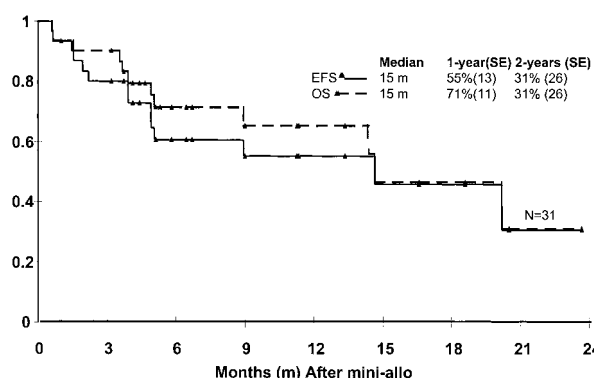


Fig 1. Actuarial and overall survival and event-free survival after mini-allografts.

ATs) allografted was statistically significant for EFS ($P = .005$) and OS ($P = .01$) (Fig 3).

Standard Allografts Versus Mini-Allografts

The comparison of patients' characteristics receiving standard allografts versus mini-allografts is provided in Table 1. Patients receiving mini-allograft were significantly older than those receiving a standard conditioning regimen (median age, 56 v 46 years; $P < .0001$). Many mini-allograft recipients had additional high-risk features, two patients had renal failure and were dialysis dependent at time of mini-allograft, and four had additional MDS clones with relapsed MM. Ninety-seven percent ($n = 30$) of the mini-allograft recipients had chromosomal abnormalities v 62% ($n = 52$) in the standard allograft patients ($P < .0001$). Chromosome 13 abnormalities were detected in 68% in the mini-allograft patients v 35% in the standard allograft patients ($P = .003$). Time from last AT to allograft was comparable in the two groups, whereas time from diagnosis was longer in the mini-allograft patients. Conditioning regimens for the standard allotransplant patients were

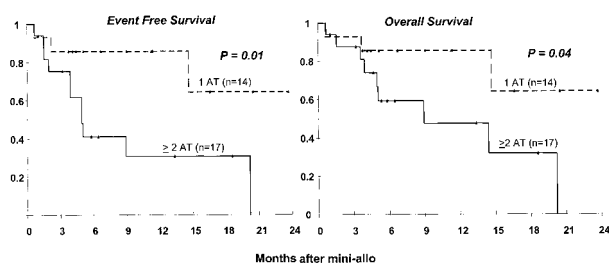


Fig 2. Actuarial survival after mini-allografts according to the number of prior autotransplants. Kaplan-Meier curves show significantly better EFS and OS among patients who received one AT prior to mini-allograft than those who received two or more ATs.

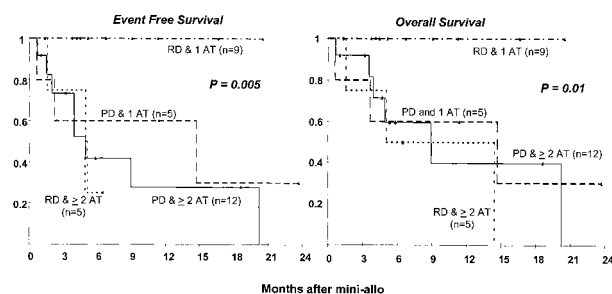


Fig 3. Actuarial survival after allografts according to number of prior autotransplants and disease status. Patients with responsive disease (RD; including CR, nCR, and PR) receiving one AT before mini-allograft had significantly better EFS and OS compared with patients with progressive disease (PD) and/or ≥ 2 ATs.

mostly TBI based and variable regimens for GVHD prophylaxis were used. Donors for the standard allograft included 14 unrelated and 79 matched sibling donors. Fifteen patients received peripheral-blood stem cells and 78 patients received bone marrow; of the marrow group, 33 had T-cell-depleted grafts.

When outcome of the mini-allograft recipients was compared with those who received standard allografts, TRM in the first 100 days was significantly lower in the mini-allograft than in the standard allograft patients (three of 31 [10%] v 27 of 93 [29%], $P = .03$, respectively). Moreover, even with the relatively short follow-up, mini-allograft patients tend to show a lower mortality during the first year ($P = .09$); the difference is especially pronounced ($P = .05$) for the subset of patients who received one AT (Fig 4). After adjusting for significant clinical variables in the multivariate model, standard allograft continued to be the only variable that negatively affected OS (hazard ratio = 2.85; $P = .007$).

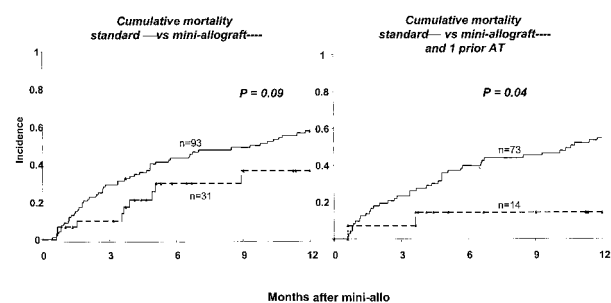


Fig 4. First-year mortality in standard allografts and mini-allografts. (A) First-year mortality in the standard allograft (n = 93) and mini-allograft (n = 31) patients. (B) First-year mortality in standard allograft (n = 73) and mini-allograft (n = 14) patients with one prior AT.

DISCUSSION

Our study shows that a low-intensity conditioning regimen is sufficient to establish durable and stable engraftment with excellent disease control even in elderly, heavily treated MM patients. These patients were not eligible for conventional allotransplant conditioning regimens because of age, comorbid conditions, and/or extent of prior therapy. All our patients had received at least one prior AT except for one patient who presented with severe MDS and had received prior fludarabine therapy. It is unlikely that our regimen will grant full donor chimerism in immunologically competent patients with limited and nonintensive prior chemotherapy.

Although the number of CD34⁺ cells required to establish engraftment has not been evaluated in our study, we used 5×10^6 CD34⁺ cells as a target dose, with incremental increase in DLI dose to convert the patients to full donor chimeras as described previously. Chimeric data showed that 80% of the patients had full donor hematopoiesis at day 30 and 90% at day 100, making it difficult to assess the role of mixed chimerism in tolerance induction to donor alloantigens and thereby decreasing the incidence of GVHD.

Early TRM (10%) for the mini-allografts especially when compared with standard allogeneic transplant (29%) was low. The main nonhematologic toxicity observed at a high rate (eight patients [26%]) was diffuse interstitial pneumonitis, which contributed to the death of three patients (two early and one late TRM). The cause is likely to be multifactorial; however, pulmonary toxicity has been reported after high-dose MEL, which is a concern because some of our patients had received cumulative doses of MEL in excess of 600 mg/m^2 .²⁰

Despite limited tissue damage and an attenuated cytokine storm (both are known GVHD-inducing factors), acute GVHD was observed with a high frequency in our patients (58%) and was associated with significant infectious complications, which was the main cause of late TRM (after day 100) in six patients. Because of this high incidence of acute GVHD, the preemptive DLI in our study initially given to all patients was subsequently limited to patients with either residual disease or mixed chimerism. We also extended the duration of posttransplant immunosuppressive therapy to day 120. To decrease the incidence and severity of GVHD, T-cell depletion was attempted in two patients. One rejected the graft after receiving a low (1.5×10^6 cells) CD34⁺ cell dose; the second patient who received 5×10^6 cells did well with no GVHD in the first 100 days. The incidence of chronic GVHD and its effect on the quality of life remains to be determined. However, the fact that this complication developed in 10 of the long-term survivors is of concern.

Future endeavors to separate subsets of T cells responsible for GVHD and GVM await further investigation.²¹⁻²³

Patients receiving major ABO-mismatched grafts ($n = 7$) had a higher rate of mixed chimerism at day 30 (four of seven [57%]) than ABO matched and minor mismatched patients (two of 24 [8%]). Of the major ABO mismatches, two patients rejected their allografts; these two patients also received the lowest CD34⁺ cell dose per kilogram (1.5 and 3.2×10^6 CD34⁺ cells/kg). One patient developed pure red cell aplasia, which is most likely a result of the survival of recipient B cells. He received CD20 monoclonal antibody (rituximab) with reconstitution of donor erythropoiesis. This complication may be avoided by targeted B-cell depletion via preemptive inclusion of rituximab as part of the conditioning regimen for ABO major mismatched recipients.

Even with advanced chemorefractory or high-risk disease of most of our patients at the time of mini-allograft, a significant proportion (61%) achieved CR/nCR. These impressive results followed full donor chimerism and were in all cases associated with GVHD and, therefore, has to be attributed to a powerful GVM effect. These immunocompetent donor lymphocytes responsible for GVM effects seem to be inseparable from those associated with GVHD. This is in contrast to other diseases (eg, chronic myelogenous leukemia), where allogeneic responses occurred without developing clinical GVHD.

As expected, patients with responsive disease before mini-allograft did especially well after allografts. The procedure seems to be less effective for patients with PD. Patients who received one AT to achieve maximal tumor reduction before mini-allograft had less toxicity and benefited the most, with an impressive 100% EFS and OS up to 2 years of follow-up. Because of the short follow-up, the durability of these antimyeloma effects cannot be assessed.

Caution should be exercised in interpreting the analysis comparing mini-allograft and standard allogeneic transplant. With a short observation period and small number of patients, we have shown that TRM in the first 100 days is significantly lower. However, no data are available to comment on the incidence and severity of long-term complications or to establish the overall efficacy of mini-allografts versus standard transplant.

In summary, we have shown that sufficient immunosuppression can be achieved with nonmyeloablative MEL-based conditioning to establish full donor engraftment in high-risk MM patients who had at least one prior AT. The regimen allowed us to expand this therapy to elderly patients. The preemptive DLI maximized GVM effects that consistently followed the development of GVHD, the most significant complication in the study. GVM effect has to be responsible for these responses because the majority of the patients were refractory to or had relapsed after multiple chemotherapy regimens including steroids and AT. Patients who received only one AT seem to have the best outcome. Our study suggests that newly diagnosed patients with poor prognosis based on cytogenetics, who achieve minimal residual disease after AT, and have a suitable HLA-matched donor should be considered for early mini-allograft because their disease-free survival with AT is expected to be brief. Future studies will focus on minimizing the risk of GVHD by increasing the duration of GVHD prophylaxis and controlling the T-cell doses in the graft.

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