**Nonmyeloablative allografting for newly diagnosed multiple myeloma: the experience of the Gruppo Italiano Trapianti di Midollo**

**Non-myeloablative Allografting for Newly Diagnosed Multiple Myeloma: the Experience of the Gruppo Italiano Trapianti di Midollo**

Benedetto Bruno,[1] Marcello Rotta,[1] Francesca Patriarca,[2] Daniele Mattei,[3] Bernardino Allione,[4] Fabrizio Carnevale-Schianca,[5] Roberto Sorasio,[1] Alessandro Rambaldi,[6] Marco Casini,[7] Matteo Parma,[8] Pasqua Bavaro,[9] Francesco Onida,[10] Alessandro Busca,[11] Luca Castagna,[12] Edoardo Benedetti,[13] Anna Paola Iori, [14] Luisa Giaccone,[1] Antonio Palumbo,[1] Paolo Corradini,[15] Renato Fanin,[2] David Maloney,[16] Rainer Storb,[16] Ileana Baldi,[17]
Umberto Ricardi,[18] and Mario Boccadoro.[1]

From the [1]Division of Hematology at the S. Giovanni Battista Hospital, University of Torino; [2]Division of Hematology, Department of Clinical and Morphological Researches, University of Udine; [3]Division of Hematology at the S. Croce e Carle Hospital, Cuneo; [4]Division of Hematology at the SS. Antonio e Biagio Hospital, Alessandria; [5]Division of Oncology, IRCC, Candiolo; [6]Division of Hematology at the Ospedali Riuniti, Bergamo; [7]Division of Hematology at the Regional Hospital, Bolzano; [8]Division of Hematology at the S. Gerardo Hospital, University of Milano, Bicocca, Monza; [9]Division of Hematology at the Ospedale Civile, Pescara; [10]Division of Hematology at the Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milano; [11]Division of Hematology at the S. Giovanni Battista Hospital, Torino; [12]Department of Medical Oncology and Hematology at the Istituto Clinico Humanitas, Rozzano, Milano; [13]Division of Hematology at the S. Chiara Hospital, University of Pisa; [14]Division of Hematology, University La Sapienza, Roma; [15]Division of Hematology at the Istituto Nazionale Tumori, University of Milano; [16]Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA, USA; [17]Unità di Epidemiologia dei Tumori at the S.G.B. Hospital and CPO Piemonte, Italy. [18]Radiation Oncology Unit at the S. Giovanni Battista Hospital, University of Torino

***Running Head*** Non-myeloablative Allografting for Myeloma

***Key words*** Non-myeloablative transplantation; allogeneic transplantation; multiple myeloma; graft-vs.-host disease; new drugs

***Counts*** *Text words 3983*; abstract words 200; tables/figures 7; references 50

***Scientific category*** Transplantation

***Correspondence*** Benedetto Bruno, M.D., Ph.D., Divisione Universitaria di Ematologia, Azienda Ospedaliera San Giovanni Battista, Via Genova 3, 10126, Torino, Italy.
Tel. +390116336107 Fax +390116963737 E-mail: benedetto.bruno@unito.it

**ABSTRACT**

Despite recent advances, allografting remains the only potential cure for myeloma. From July 1999 to June 2005, 100 newly diagnosed patients younger than 65 years were enrolled in a prospective multi-center study. First-line treatment included vincristin, adriamycin and dexamethasone (VAD)-based induction chemotherapy, a cytoreductive autograft (melphalan 200 mg/m2) followed by a single dose of non-myeloablative total body irradiation and allografting from an HLA-identical sibling. Primary endpoints were overall (OS) and event-free (EFS) survivals from diagnosis. After a median follow up of 5 years, OS was not reached and EFS was 37 months. Incidences of acute and chronic graft-vs.-host disease (GHVD) were 38% and 50%, respectively. Complete remission (CR) was achieved in 53% of patients. Profound cytoreduction (CR or very good partial remission) prior to allografting was associated with achievement of post-transplant CR (HR 2.20, p=0.03) and longer EFS (HR 0.33, p<0.01). Conversely, development of chronic GVHD was not correlated with CR or response duration. This tandem transplant approach allows prolonged survival and long-term disease control in patients with reduced tumor burden at the time of allografting. We are currently investigating the role of "new drugs" in intensifying pre-transplant cytoreduction and post-transplant graft-vs.-myeloma effects to further improve clinical outcomes. (ClinicalTrial.gov, NCT-00702247)

## INTRODUCTION

Despite remarkable recent advances in its treatment, multiple myeloma remains incurable.[1] Allografting is still regarded as the only potential cure on account of its well-documented graft-vs.-myeloma effect observed in a subset of patients.[2-5] However, its use remains controversial especially in newly diagnosed patients.

In the late '90s, the introduction of reduced intensity / non-myeloablative conditionings greatly renewed the interest in allografting in particular for diseases such as myeloma where the transplant-related mortality (TRM) with conventional transplant regimens had been unacceptably high.[5-7] Combining the cytoreductive effect of a high-dose melphalan-based autograft with the graft-vs.-myeloma effects of a non-myeloablative allograft reduced TRM even in elderly, medically unfit myeloma patients.[8,9]

Our recent comparison between autografting and non-myeloablative allografting showed that the latter resulted in longer overall and event-free survivals in newly diagnosed patients younger than 65 years.[10] Preliminary reports from other groups have confirmed our findings.[11,12] Here, we report on an extended experience consisting of 100 newly diagnosed myeloma patients enrolled in a prospective clinical trial (ClinicalTrial.gov, NCT-00702247) and treated with non-myeloablative allografts as part of their first-line treatment at fifteen Italian Bone Marrow Transplantation Units of the Gruppo Italiano Trapianti di Midollo Osseo (GITMO).

## METHODS

***Patients and donors*** From July 1999 to June 2005, 100 newly diagnosed myeloma patients younger than 65 years were enrolled in a prospective multi-center trial. Informed consent was obtained upon enrolment. The protocol was approved by the Institutional Review Boards of the participating Centers according to the Declaration of Helsinki.

Inclusion criteria included: diagnosis of untreated Durie & Salmon stage IIA-IIIB multiple myeloma or stage I progressed to require therapy; age < 65 years; Karnofsky performance status

>60%; presence of an HLA-identical sibling donor eligible for peripheral blood stem cell (PBSC) donation. Exclusion criteria included: prior treatment for myeloma; abnormal cardiac function and chronic respiratory disease defined as systolic ejection fraction < 35% and carbon monoxide diffusing capacity < 40% of predicted or need of continuous supplemental oxygen, respectively; serum bilirubins > twice normal, and ALAT and/or ASAT > four times normal; poorly controlled hypertension; pregnancy, seropositivity for HIV. Patients with active non-hematologic malignancies, except non-melanoma skin cancers, or who were less than 5 years from the achievement of complete remission with a >20% risk of disease recurrence were also excluded.

Sibling donors < 75 years of age were serologically matched for HLA-A , B and C antigens, and by high resolution typing for HLA-DRB1 and DQB1 alleles. Donors gave consent to granulocyte-colony stimulating factor (G-CSF) administration and to leukapheresis for PBSC collections. Pregnant women, identical twins, HIV-positive individuals and potential donors with known allergy to G-CSF were excluded from donation.

***Induction therapy, PBSC mobilization and autografting*** Initial treatment plan included induction chemotherapy, mainly consisting of 2-3 courses of vincristine-adriamycin-dexamethasone (VAD)-based regimens, followed by PBSC mobilisation and harvest (target of at least 2 x $10^6$ CD34 cells/kg) after 1 or 2 cycles of cyclophosphamide, 3-4 g/$m^2$, with or without paclitaxel, 250 mg/$m^2$, and G-CSF, 10 μg/kg given i.v. or subcutaneously. After at least one month from PBSC collection, autografting consisted of melphalan, 200 mg/$m^2$, on day –2, and cryopreserved PBSC infusion on day 0. Patients received G-CSF, 5 μg/kg, from days 1 or 3 until neutrophil counts >1000/μl were achieved.

***Donor mobilisation*** HLA-identical sibling donors, mean age 54 (range 32-69) years, were mobilised with G-CSF, 16 μg/kg/day (day –4 to 0), with aphereses on days –1 and 0. PBSC harvested on day –1 were stored overnight at 4ºC and freshly infused with the day 0 collection. The entire collections were infused and a minimum target of 5 x $10^6$ CD34-positive cells/kg of recipient body weight was recommended. No upper limit was defined.

***Non-myeloablative allografting*** Upon recovery from autografting, defined as resolved mucositis, no evidence of cytomegalovirus (CMV) reactivation or disease, and no need for intravenous medications, planned range of 2-4 months, patients were conditioned for allografting with a single dose of non-myeloablative 200 cGy total body irradiation (TBI) on day 0. Postgrafting immunosuppression consisted of mycophenolate mofetil, 15 mg/kg orally BID from the evening of day 0 until day 27, and cyclosporine, 6.25 mg/kg orally BID from day -3 or 1.5 mg/kg iv BID from day -1 through day 80 and then tapered.[9,10] No maintenance/consolidation therapy was allowed by protocol after non-myeloablative allografting.

***Analyses of chimerism*** Chimerism analyses of peripheral blood T cells, granulocytes and unfractionated marrow were carried out at days 28, 56, 180, 360 after allografting and every six months thereafter or as clinically indicated with fluorescence in situ hybridization (FISH) in sex-mismatched pairs or polymerase chain reaction (PCR)-based analyses of polymorphic microsatellite regions in sex-matched pairs as previously described.[9,10]

***Chromosomal abnormalities*** 13q deletion (del13) was analysed by interphase FISH techniques on freshly purified bone marrow plasma cells as previously described.[13]

***Supportive care and GVHD grading*** Following allografting, all patients received standard prophylaxis against bacterial and fungal infections; herpes simplex and varicella-zoster virus reactivation; and Pneumocystis carinii. CMV reactivation was monitored through levels of CMV antigenemia and/or serum CMV DNA levels and treated with ganciclovir or foscarnet as clinically indicated. Standard criteria were used for diagnosis and grading of acute and chronic GVHD.[14,15]

***Salvage therapy*** Standard chemotherapy and/or thalidomide- or bortezomib-containing regimens, as per Institutional guidelines of the participating Centers, with/without donor lymphocytes infusions (DLI) were allowed to treat progression or relapse post-transplant. DLI were administered in the absence of GVHD clinical manifestation and after a rapid taper and discontinuation of the immunosuppression.

***Disease response*** Response was evaluated prior to each treatment, monthly for the first six months following allografting and at least every three months thereafter or as clinically indicated. Response criteria were defined according to the International Uniform Response Criteria for multiple myeloma.[16] Complete remission (CR) required absence of serum monoclonal immunoglobulins and/or Bence-Jones proteinuria by electrophoresis and immunofixation, less than 5% plasma cell infiltration in bone marrow aspirates, absence of soft tissue lesions and no increase in size or number of osteolytic lesions. Very good partial remission (VGPR) was defined as detection of serum monoclonal immunoglobulins and/or Bence-Jones proteinuria by immunofixation but not by electrophoresis or at least 90% reduction in Bence-Jones proteinuria with excretion lower than 100 mg /24-hour, and no increase in size or number of osteolytic lesions. Partial remission (PR) was defined as >75% reduction in the levels of serum monoclonal immunoglobulin, at least 90% reduction in 24-hour urinary light chain excretion, and no increase in size or number of lytic bone lesions. Patients with less than a PR after induction chemotherapy or autografting were considered refractory, whereas the disease was considered stable if no response, meeting the criteria of CR, VGPR or PR, was observed after allografting. Response criteria had to be met on at least two consecutive occasions at least six weeks apart. Progressive disease (PD) was considered an increase in serum monoclonal proteins or urine light chains of a least 25% in patients with refractory or stable disease, whereas relapse was considered as the reappearance of bone marrow infiltration, serum monoclonal immunoglobulins or urine light chains or new bone lesions in patients in previous CR, or a 25% increase in any disease marker for patients in prior PR.

***Statistical analysis*** Primary endpoints of the study were overall (OS) and event-free survivals (EFS) from diagnosis according to the intention-to-treat principle and in patients who completed the program. Secondary endpoints included transplant-related toxicity and TRM and incidence of acute and chronic GVHD. OS and EFS were calculated according to the Kaplan-Meier technique from the date of diagnosis until death from any cause and from the date of diagnosis until the date of first relapse or progression or of death from any cause, respectively.[17] Deaths not related to myeloma or

to non-hematological malignancies were classified as deaths from transplant-related toxicity. Moreover, an estimation of the probability that a patient was alive in the original remission or in a subsequent remission after salvage treatment was carried out by the "Current Progression Free Survival" (CPFS) method as described by Klein et al.[18] Estimates of the incidence of acute and chronic GVHD, TRM and disease-related mortality were calculated with the cumulative incidence method described by Gooley et al, in which risks of death in CR and of relapse were considered as competing risks.[19] The individual effect of patient characteristics on time from allografting to five different events (relapse/progression, death, CR after transplant, chronic extensive GVHD and acute grade II-IV GVHD) were evaluated using Cox's proportional-hazards regression models with the Wei-Lin-Weissfeld estimators.[20-23] Proportional hazard assumptions were checked with the Grambsh and Therneau test.[24] Predictors were chosen for each outcome in the light of potential clinical impact and sample size as follows: prognostic role of age, isotype of myeloma protein, International Staging System (ISS) score, disease in remission, defined as VGPR or CR, at the time of allografting and comorbidity index ≥ 3 for both OS and EFS; chronic GVHD, as time-dependent covariate, age, isotype of myeloma protein, ISS, disease in remission at the time of allografting for both the achievement of CR and the risk of relapse/progression post-allografting; effects of CD34-pos cells and CD3-pos T cells infused, age and donor gender for the risk of developing acute and chronic GVHD.[25-26] Results are presented as hazard ratios (HR) with corresponding 95% confidence intervals (95% CI) and p-values. SAS 8.2 statistical software (SAS Institute, Cary, NC) and R.2.1.0 software, package cmprsk were used.

## RESULTS

***Patients*** Patient characteristics are shown in Table 1. Overall, 100 patients with at least one HLA-identical sibling were enrolled at fifteen Italian Bone Marrow Transplantation Units. Ninety-six/100 (96%) completed the protocol whereas 4 did not because of consent withdrawal (no=2); infectious complications after the autograft (no=1); ineligible donor at pre-transplant work-up

(no=1). Fifty-two, also described in an earlier report, have been included in this series after updating their follow-up.[10]

***Engraftment and Response*** Ninety-six allografts were carried out at a median of 90 (range 44-396) days after the autograft. One patient underwent the allograft 13 months after the autograft because of viral encephalitis. The median numbers of CD34+ cells and CD3+ T cells infused were 7.5 x $10^6$/Kg (range, 2.6-26.4 x$10^6$) and 3.2 x $10^7$/Kg (range, 0.7-18.9 x$10^7$) recipient body weight, respectively. All patients readily achieved engraftment with median percentages of donor cells at 1 month post transplant of 97%, 97%, and 97% among blood T-cells, granulocytes and unfractionated bone marrow respectively.

Forty-eight/100 patients had chemo-sensitive disease (2 CR, 6 VGPR, and 40 PR) at the time of autografting, while, among the 96 patients who completed the program, 6 (6%) were in CR, 29 in VGPR (30%) and 38 (40%) in PR at the time of allografting. Overall, after the allograft, 51 (53%) patients achieved CR at a median of 4 (range 1-45) months, 15 (16%) VGPR and 21 PR (22%), giving an overall response of 91%. With a median follow-up of 5 (range 2.3-8.4+) years from diagnosis and 4.3 (1.8-7.4+) from allografting, 14/51 (27%), 10/15 (67%) and 12/21 (57%) patients had relapsed from CR, VGPR and PR respectively*, giving an overall relapse rate of 41% (36/87). Overall response and relapse rates for the entire cohort of patients were 88% (88/100) and 44% (39/88) respectively.* Disease-related mortality was 5.2% at 2 years (95% CI: 0.8-9.6%); 20.5 at 5 years (95% CI: 11.7-29.3%) (Figure 1-C).

Formattato

***Salvage Therapy*** Thirty-six patients were treated for disease relapse and 6 for progression of stable disease. First-line salvage therapy consisted of bortezomib- or thalidomide-containing regimens in 12 and 16 patients, respectively, and standard chemotherapy and/or radiotherapy in 8. Moreover, 9 of these patients received DLI as consolidation therapy. Five patients received DLI alone. In one patient, the immunosuppression was tapered and eventually discontinued without further therapy. Overall, 6/42 (14%) patients obtained a CR and 13/42 (31%) a PR. Five patients experienced a second relapse.

Only 4 patients received DLI for post-transplant stable disease. Overall, among the 18 treated with DLI, only 1 (7%) patient reached CR and 3 (17%) others PR.

***Transplant-related Toxicity*** Thirty-six patients developed grade II-IV acute GVHD at a median of 41 (range 20-115) days. This was grade IV in 3 patients. Cumulative incidence of grade II-IV and grade IV GVHD was 38% and 3%, respectively (Figure 1-A). Forty-seven (50%) of 94 patients with a follow-up of at least 120 days developed extensive chronic GVHD. Overall, 53/85 patients, 27/73 and 10/36 remained on immunosuppression at 1, 2 and 4 years post-allografting respectively. Six/53 patients who developed acute GVHD had a flare after initial therapy that required an immunosuppression taper longer than one year. Most patients had a Karnofsky performance status of 90-100% despite immunosuppression. One patient in CR with severe bronchiolitis obliterans successfully underwent lung transplantation. Twenty-nine (30%) patients have died: 15 (16%) from disease progression, 11 (11%) from transplant-related toxicity and 3 from another malignancy. TRM was due to progressive encephalopathy (n=1), complications associated with acute or chronic GVHD (n=8), and HUS-TTP syndrome (n=2). Overall transplant-related mortality was 11.4% (95% CI: 4.8-17.2%) (Figure 1-B). By multivariable analysis, an increasing number of CD34+ cells infused, but not CD3+ T cells, was associated with a significant risk of developing acute GVHD, but not chronic GVHD (HR 1.12, CI 95% 1.04-1.21, $p<0.01$ and HR 1.03, CI 95% 0.94-1.13, $p=0.55$) (Table 2). Furthermore, the development of chronic GVHD was not significantly associated with either the subsequent achievement of CR or disease relapse/progression (HR 0.80, CI 95% 0.43-1.47, $p=0.47$ and HR 0.85, CI 95% 0.51-1.42, $p=0.54$) (Table 2).

***Outcome*** By the intention to treat principle, after a median follow up of 5 (range 0.7-8.4+) years from diagnosis, median OS was not reached and median EFS was 2.9 (range 2.4-4.3) years (Figure 2-A, 2-B). Among the 96 patients who completed the program, after a median follow up of 5 (range 2.3-8.4+) years from diagnosis, median OS was not reached whereas median EFS was 3.1 (range 2.6-4.5) years (Figure 2-C, 2-D). No differences in both the updated OS and EFS between

the previously reported cohort of 52 patients[10] and the newly described 44 were observed (HR 0.58, CI 95% 0.28-1.2, p=0.14 and HR 0.93, CI 95% 0.55-1.56, p=0.78, respectively). Furthermore, the probability of a patient being alive in first remission or in a subsequent remission due to salvage therapy is illustrated in Figure 3.

Multivariable analyses for OS and EFS are reported in Table 3. Irrespective of myeloma isotype, ISS, comorbidity index ≥ 3, disease in remission was significantly associated with longer OS and EFS (HR 0.20, CI 95% 0.06-0.67, p=0.01 and HR 0.33, CI 95% 0.17-0.65, p<0.01). Age, as a continuous variable, was also significantly associated with both OS and EFS (HR 1.06, CI 95% 1.00-1.14, p=0.05 and HR 1.07, CI 95% 1.02-1.12, p<0.01, respectively). Chromosome 13 abnormalities [del(13)] were studied in 39/96 (41%) patients: 13/39 (33%) showed del(13) whereas 26/39 (67%) did not. There was no significant difference in median OS between the two cohorts of patients (not reached vs. 4.3 years, p=0.18) whereas EFS was better in patient without del(13) (4.3 vs. 2.2 years, p=0.01) (Figure 4-A, 4-B).

## DISCUSSION

Progress in myeloma treatment has been impressive in the past ten years with the introduction of high-dose melphalan followed by autologous transplantation and, recently, through the identification of new agents with molecular targets such as thalidomide, lenalidomide and bortezomib.[27-32] OS has been significantly prolonged especially in good risk patients, whereas eradication of the disease seems unlikely. Conversely, in the light of a well-documented graft-vs.-myeloma effect, allografting may be curative in a subset of patients.[5,33,34] However, its role has never been thoroughly investigated. First, the high transplant-related toxicity associated with myeloablative conditionings has severely limited its application; second, the retrospective nature of several studies with strong patient selection bias and the lack of large prospective controlled trials have not allowed definitive conclusions.[7] One of the strengths of our study is the rigid enrolment at diagnosis of untreated myeloma patients who underwent the same VAD-based induction treatment

before the autologous cytoreductive transplant. This strategy meant that they were all treated uniformly and any statistical bias was greatly reduced. By contrast, prospective studies which include allografting as part of the up-front treatment regardless of the induction therapy inevitably result in bias that may highly affect the clinical outcomes.

In the present study, median OS was not reached after a follow-up of 5 years. Before the era of "new drugs", after a median follow-up of 75 months, Attal et al. reported median OS of 48 and 58 months after one and two autologous transplants respectively.[28] Barlogie et al.[35] reported that 17% of the patients enrolled in the Total Therapy 1 trial were alive at 15 years, 7% event free, after a median follow-up of 12 years. [29] More recently, with a follow-up of 7 years, the thalidomide arm of Total Therapy 2 has appeared to further improve clinical outcomes. [35]

Overall, 53% (51/96) of patients reached CR, 73% (37/51) of whom are in continuous CR, including molecular remissions (Ladetto M., personal communication), prelude to a cure, with a follow-up extending to 8 years. Thus, the depth of response was crucial for prolonged response in our study (Table 3).[36] Given the high rate of CR obtained without the use of so-called "new drugs", it is imperative to thoroughly explore their role in the setting of allografting. Graft-vs.-myeloma effects and "new drugs" with molecular targets, in fact, are by no means mutually exclusive. Bortezomib and thalidomide have already been shown to re-induce remissions in patients who relapsed following allografting.[37-34] Anti-myeloma activity has also been shown at relapse in the series of patients reported in this study (Figure 3). We are currently investigating the role of lenalidomide in reducing the tumor burden before and enhancing graft-vs-myeloma effect after transplant. Maintenance therapy may lead to a significant increase in response rates and prolonged response duration. Furthermore, this strategy may also overcome the higher risk of relapse in patients with poor prognostic factors. [40]

Overall, non-relapse mortality was 11%. GVHD and its complications accounted for most TRM. Its incidence, however, may be further reduced as progress is made in the understanding of its pathogenesis.[41] Chronic GVHD has often been associated with longer response duration and better OS.[42] In our study, however, its development did not correlate with either subsequent achievement of CR or response duration (Table 2). In a subset of patients, therefore, a graft-vs.-myeloma effect may be distinct from detrimental chronic GVHD or associated with subclinical graft-vs.-host reactions. The potential biological effects of the number of donor CD34-pos cells infused have been debated in several studies.[43,44] Interestingly, we noted that an increasing number of CD34-pos cells infused, but not CD3-pos T cells, was significantly correlated with a higher risk of developing acute GVHD (Table 2). An upper limit of CD34-pos cells infused was not included in our trial. Evaluation of their number in a larger series of patients may help to set a range that allows consistent donor engraftment while reducing the risk of acute GVHD.

It is widely assumed that chromosomal abnormalities are important prognostic factors for both OS and EFS.[45] Garban et al. reported OS and EFS of 35 and 31.7 months respectively, after a median follow-up of 2 years, in high risk newly diagnosed myeloma patients, with either elevated β-2-microglobulin levels or presence of del(13), who received reduced-intensity allografts.[46] Perhaps because the conditioning with high-dose anti-thymocyte globulin may have attenuated graft-vs.-myeloma effects, a survival benefit with allografting as compared to melphalan-based autografting was not observed.[47] In another comparison, the advantage of having an HLA identical sibling, therefore the chance of undergoing an allograft, as compared to not having an HLA-identical sibling was not offset by the presence of del(13).[10] This finding, however, did not imply that in patients given allografts, del(13) might not have had a prognostic role. In the present series, del(13) appeared to significantly affect EFS (4.3 vs. 2.2 years, p=0.01), but not OS (not reached vs. 4.3 years, p=0.18). The data reported in all these studies should, however, be evaluated with larger and more comprehensive analyses that include a complete spectrum of the chromosomal

abnormalities associated with myeloma rather than a single abnormality. Recent reports clearly showed that del(13) alone did not affect OS after transplant unless it was associated with other abnormalities such as del(17) or t(4;14).[47]

Whether an allograft should be offered as part of first-line treatment plan or as salvage therapy for refractory or relapsed patients is a matter of debate.[48] Though allografting with reduced intensity/non-myeloablative conditionings has evolved into a less toxic procedure, new methods to further reduce toxicity while maintaining graft-vs.-myeloma effects are being investigated. In our experience, the use of low-dose TBI conditioning regimens up-front proved significantly more effective in terms of graft-vs.-myeloma effects than waiting with transplant until relapse.[49] Poor response to post-transplant donor lymphocyte infusions at relapse, without prior cytoreduction, was also observed. These might be due to an antigen expression profile of potential targets for allogeneic cytotoxic T cells that progressively changed. For instance, Siegel et al. identified HLA-A*0201-presented T cell epitopes derived from the oncofetal antigen-immature laminin receptor protein in hematological malignancies including myeloma.[50] Expression of these antigens on myeloma cell was lost over time. Rosinol et al recently reported on the PETHEMA study. [12] Patients who did not achieve at least near-CR after a first autograft were randomized to receive either a second autograft or an allograft after a reduced-intensity-conditioning in the light of the presence/absence of an HLA-identical sibling donor. There was a significantly higher incidence of CR and a longer progression-free survival in patients treated with an allograft. However, there was also a higher TRM and no statistical difference in EFS and OS. The Authors concluded that, although the progression-free survival plateau was encouraging, the procedure should be investigated in prospective clinical trials. Though different in design, the findings of the PETHEMA study are not conflicting with our trial. In particular, we clearly observed that disease response at the time of the allograft was significantly associated with post-transplant EFS and OS (Tables 2 and 3).

In summary, our findings suggested that allografting was effective in the treatment of newly diagnosed myeloma patients. The combination of graft-vs.-myeloma effects with "new drugs" should be clinically evaluated in well-designed phase III clinical trials where control groups should include patients treated with new agents with potent anti-myeloma activity with/without autografting. Moreover, stratification of patients by prognostic factors, especially chromosomal abnormalities, is imperative to determine those who may most benefit from a "tandem transplant approach".

## ACKNOWLEDGEMENTS

The following also contributed to the manuscript: Antonio Capaldi,[5] Giovannino Ciccone,[17] Paolo Di Bartolomeo,[9] Michele Falda,[11] Andrea R. Filippi,[18] Robin Foà,[14] Giorgio Lambertenghi-Deliliers,[10] Alessandro Levis,[4] Massimo Massaia,[1] Vittorio Montefusco,[15] Nicola Mordini,[3] Mario Petrini,[13] Enrico Pogliani,[8] Brenda M. Sandmaier,[16] Barry E. Storer [16]

Contributions: G.C, B.E.S., analysed data; A.C., P.D.B., M.F., A.R.F., R.F., G.L.D., A.L., M.M., V.M., N.M., M.P., E.P., B.M.S. made substantial conceptual contributions.

This research was supported in part by Progetti di Ricerca ex-60%, Ministero dell'Università e della Ricerca Scientifica (M.I.U.R.); Regione Piemonte: Ricerca Finalizzata 2005 (Progetto Clinico-scientifico e di Coordinamento Regionale), 2006, 2007; Compagnia di San Paolo; Fondazione Cassa di Risparmio di Torino (C.R.T.) and Comitato Regionale Piemontese Gigi Ghirotti (Progetto Vita Vitae); Fondazione Neoplasie Sangue Onlus; grant CA78902 from the National Institute of Health, DHHS, Bethesda, MD.

Our thanks to the nurses and medical staff for caring for the patients and to the study co-ordinators who collected the trial and follow-up data.

## AUTHORSHIP

***Contributions*** B.B and M.B. designed and directed the study, edited the manuscript; I.B. performed statistical analyses, reviewed manuscript; U.R. participated in designing research/protocol, reviewed manuscript; M.R. and R. Sorasio contributed patients to the study, verified data, assisted in drafting manuscript; F.P., D. Mattei., B.A., F.C.-S., A.R., M.C., M. Parma, P.B., F.O., A.B., L.C., E.B., A.P.I., L.G., A.P., P.C., R. F. contributed patients to the study, reviewed manuscript; D. Maloney, R. Storb contributed to study design, reviewed manuscript.

***Conflict of interest disclosure*** The authors declare that they have no conflict of interest

***Correspondence*** Address correspondence to Benedetto Bruno, Divisione Universitaria di Ematologia, Azienda Ospedaliera San Giovanni Battista, Via Genova 3, 10126, Torino, Italy. E-mail: benedetto.bruno@unito.it

## REFERENCES


1. Kyle RA, Rajkumar SV. Multiple myeloma. Blood. 2008;111:2962-2972.
2. Aschan J, Lonnqvist B, Ringden O, Kumlien G, Gahrton G. Graft-versus-myeloma effect. Lancet. 1996;348:346.
3. Tricot G, Vesole DH, Jagannath S, Hilton J, Munshi N, Barlogie B. Graft-versus-myeloma effect: proof of principle. Blood. 1996;87:1196-1198.
4. Le Blanc R, Montminy-Métivier S, Bélanger R, et al. Allogeneic transplantation for multiple myeloma: further evidence for a GVHD-associated graft-versus-myeloma effect. Bone Marrow Transplant. 2001;28:841-848.
5. Barlogie B, Kyle RA, Anderson KC, et al. Standard chemotherapy compared with high-dose chemoradiotherapy for multiple myeloma: final results of phase III US Intergroup Trial S9321. J Clin Oncol. 2006;24:929-936.
6. Bensinger WI, Buckner CD, Anasetti C, et al. Allogeneic marrow transplantation for multiple myeloma: an analysis of risk factors on outcome. Blood. 1996;88:2787-2793.
7. Gahrton G, Svensson H, Cavo M, et al. Progress in allogenic bone marrow and peripheral blood stem cell transplantation for multiple myeloma: a comparison between transplants performed 1983-93 and 1994-8 at European Group for Blood and Marrow Transplantation centres. Br J Haematol. 2001;113:209-216.
8. Kroger N, Schwerdtfeger R, Kiehl M, et al. Autologous stem cell transplantation followed by a dose-reduced allograft induces high complete remission rate in multiple myeloma. Blood. 2002;100:755-760.
9. Maloney DG, Molina AJ, Sahebi F, et al. Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. Blood. 2003;102:3447-3454.
10. Bruno B, Rotta M, Patriarca F et al. A comparison of allografting with autografting for newly-diagnosed myeloma. N Engl J Med. 2007;356:1110-1120

11. Carella AM, Lerma E, Nati S et al. Reduced Intensity Conditioning Regimen for Allografting Following Autografting Has Strong Anti-Myeloma Activity and Delays Disease Progression Compared to Tandem Autografting. Blood (ASH Annual Meeting Abstracts). 2006;108:abstract 51
12. Rosinol L, Pérez_Simon JA, Sureda A, et al. A prospective PETHEMA study of tandem autologous transplantation versus autograft followed by reduced-intensity conditioning allogeneic transplantation in newly diagnosed multiple myeloma. Blood.
13. Schmidt-Wol IG, Glasmacher A, Hahn-Ast C, et al. Chromosomal aberrations in 130 patients with multiple myeloma studied by interphase FISH: diagnostic and prognostic relevance. Cancer Genet Cytogenet. 2006;167:20-25.
14. Przepiorka D, Weisdorf D, Martin P, et al. Consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825-828.
15. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. Semin Hematol. 1991;28:250-259.
16. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006;20:1467-1473. Erratum in: Leukemia. 2006;20:2220. Leukemia. 2007;21:1134.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457-481.
18. Klein JP, Szydlo RM, Craddock C, et al. Estimation of current leukaemia-free survival following donor lymphocyte infusion therapy for patients with leukaemia who relapse after allografting: application of a multistate model. Stat Med. 2000;19:3005-3016
19. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695-706.
20. Cox DR. Regression models and life-tables (with discussion). J R Statist Soc. 1972;24:187-220.

21. Wei DY, Lin LJ and Weissfeld L. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. Journal of the American Statistical Association. 1989; 84:1065-1073.
22. Lee LJ, Wei EW and Amato DA. Cox-type regression analysis for large number of small groups of correlated failure time observations. In: Klein JP and Goel PK, eds. Survival analysis: state of the art. Dordrecht, Kluwer Academic; 1992:237–247.
23. Lin DY. Cox regression analysis of multivariate failure time data: the marginal approach. Stat Med. 1994; 13:2233-2247.
24. Grambsh, P and Therneau, T. Proportional hazard tests and diagnostics based on weighted residuals. Biometrika. 1994; 81:515-526.
25. Harrell FE Jr. Regression Modeling Strategies. New York, Springer; 2001.
26. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. J Clin Oncol. 2005;23:3412-3420. Erratum in: J Clin Oncol. 2005;23:6281.
27. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348:1875-1883.
28. Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med. 2003;349:2495-2502. Erratum in: N Engl J Med. 2004;350:2628.
29. Barlogie B, Tricot GJ, van Rhee F, et al. Long-term outcome results of the first tandem autotransplant trial for multiple myeloma. Br J Haematol. 2006;135:158-164.
30. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005;352:2487-2498.
31. Dimopoulos M, Spencer A, Attal M, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med. 2007;357:2123-2132.
32. Weber DM, Chen C, Niesvizky R, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. N Engl J Med. 2007;357:2133-2142.

33. Corradini P, Voena C, Tarella C, et al. Molecular and clinical remissions in multiple myeloma: role of autologous and allogeneic transplantation of hematopoietic cells. J Clin Oncol. 1999;17:208-215.
34. Corradini P, Cavo M, Lokhorst H, et al. Molecular remission after myeloablative allogeneic stem cell transplantation predicts a better relapse-free survival in patients with multiple myeloma. Blood. 2003;102:1927-1929.
35. Barlogie Barlogie B, Pineda-Roman M, et al. Thalidomide arm of Total Therapy 2 improves complete remission duration and survival in myeloma patients with metaphase cytogenetic abnormalities. Blood. 2008;112:3115-21.
36. Dingli D, Pacheco JM, Nowakowski GS, et al. Relationship between depth of response and outcome in multiple myeloma. J Clin Oncol. 2007;25:4933-4937.
37. Kroger N, Zabelina T, Ayuk F, et al. Bortezomib after dose-reduced allogeneic stem cell transplantation for multiple myeloma to enhance or maintain remission status. Exp Hematol. 2006;34:770-775.
38. Bruno B, Patriarca F, Sorasio R, et al. Bortezomib with or without dexamethasone in relapsed multiple myeloma following allogeneic hematopoietic cell transplantation. Haematologica. 2006;91:837-839.
39. van de Donk NW, Kroger N, Hegenbart U, et al. Remarkable activity of novel agents bortezomib and thalidomide in patients not responding to donor lymphocyte infusions following nonmyeloablative allogeneic stem cell transplantation in multiple myeloma. Blood. 2006;107:3415-3416.
40. San-Miguel J, Harousseau JL, Joshua D and Anderson KC. Individualizing treatment of patients with myeloma in the era of novel agents. J Clin Oncol. 2008;26:2761-6.
41. Lowsky R, Takahashi T, Liu YP, et al. Protective conditioning for acute graft-versus-host disease. N Engl J Med. 2005;353:1321-1331. Erratum in: N Engl J Med. 2006;354:884.

42. Baron F and Storb R. The immune system as a foundation for immunologic therapy and hematologic malignancies: a historical perspective. Best Pract Res Clin Haematol. 2006;19:637-653

43. Baron F, Maris MB, Storer BE, et al. High doses of transplanted CD34+ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. Leukemia. 2005;19:822-828.

44. Baron F, Sandmaier BM, Gooley T et al. Higher Doses of Transplanted T and B Cells Are Associated with Greater Incidence of Extensive Chronic GVHD after PBSC Transplantation from HLA-Identical Sibling Donors. Blood (ASH Annual Meeting Abstracts); 2007;110:abstract 1077.

45. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myélome. Blood. 2007;109:3489-3495.

46. Garban F, Attal M, Michallet M, et al. Prospective comparison of autologous stem cell transplantation followed by dose-reduced allograft (IFM99-03 trial) with tandem autologous stem cell transplantation (IFM99-04 trial) in high-risk de novo multiple myeloma. Blood. 2006;107:3474-3480.

47. Schilling G, Hansen T, Shimoni A, et al. Impact of genetic abnormalities on survival after allogeneic hematopoietic stem cell transplantation in multiple myeloma. Leukemia. 2008;22:1250-1255.

48. Harousseau JL. The allogeneic dilemma. Bone Marrow Transplant. 2007;40:1123-1128.

49. Giaccone L, Bruno B, Rotta M, et al. Limited graft versus myeloma effect after low-dose TBI based non-myeloablative allografting employed as salvage treatment compared to first line treatment. Bone Marrow Transplant. 2006;37:abstract 0126.

50. Siegel S, Wagner A, Friedrichs B, et al. Identification of HLA-A*0201-presented T cell epitopes derived from the oncofetal antigen-immature laminin receptor protein in patients with hematological malignancies. J Immunol. 2006;176:6935-6944.

**TABLE 1**

**Patient characteristics**

| **Characteristic** | **Patients enrolled (n=100)** Number (%) | **Patients who completed program (n=96)** Number (%) |
|---|---|---|
| Male | 52 (52) | 52 (54) |
| Mean age, years (range) | 54 (30-65) | 54 (30-65) |
| Durie&Salmon Stage II | 29 (29) | 26 (27) |
| Durie&Salmon Stage III | 67 (67) | 66 (69) |
| International Staging System 2 | 22/92 (24) | 22/88 (25) |
| International Staging System 3 | 14/92 (15) | 12/88 (14) |
| Ig-G myeloma | 57 (57) | 56 (58) |
| Ig-A myeloma | 18 (18) | 18 (19) |
| Ig-D myeloma | 1 (1) | 0 |
| Bence Jones myeloma | 18 (18) | 17 (18) |
| Non-secretory myeloma | 6 (6) | 5 (5) |
| β-2-microglobulin $\geq$ 3.5 mg/dl | 33/95 (35) | 31/91 (34) |
| Albumin <3.5 g/dl | 21/95 (22) | 21/92 (23) |
| Creatinine $\geq$2 mg/dl | 11 (11) | 11 (11) |
| LDH above normal level | 17/91 (19) | 16/88 (18) |
| Presence of Ch 13 deletion | 14/43 (33) | 13/39 (33) |
| HCT-Specific Comorbidity Index $\geq$ 3 | 11 (11) | 10 (10) |

Abbreviations: LDH lactate dehydrogenase; HCT Hematopoietic Cell Transplantation

**TABLE 2**

**Cox models for achievement of complete remission, for progression/relapse and for development of acute and chronic GVHD**

| | **Multivariable Analyses** | | | |
|---|---|---|---|---|
| **Variable** | ***Achievement of CR*** | | ***Progression/relapse*** | |
| | **HR (95% CI)** | ***P-value*** | **HR (95% CI)** | ***P-value*** |
| Development of chronic GVHD° | 0.80 (0.43-1.47) | *0.47* | 0.85 (0.51-1.42) | *0.54* |
| Age* | 0.96 (0.92-1.00) | *0.05* | 1.08 (1.03-1.13) | *<0.01* |
| Ig-G Myeloma | 0.77 (0.45-1.30) | *0.33* | 0.61 (0.35-1.06) | *0.08* |
| International Staging System 3 | 0.66 (0.22-1.98) | *0.46* | 1.39 (0.69-2.79) | *0.35* |
| Disease in remission[§] at allografting | 2.20 (1.18-4.08) | *0.03* | 0.30 (0.15-0.62) | *<0.01* |
| | ***Development of Acute GVHD*** | | ***Development of Chronic GVHD*** | |
| | **HR (95% CI)** | ***P-value*** | **HR (95% CI)** | ***P-value*** |
| CD3+ cells/Kg recipient weight* | 0.98 (0.81-1.18) | *0.85* | 1.05 (0.97-1.13) | *0.23* |
| CD34+ cells/Kg recipient weight* | 1.12 (1.04-1.21) | *<0.01* | 1.03 (0.94-1.13) | *0.55* |
| Age* | 1.03 (0.98-1.09) | *0.25* | 1.05 (1.01-1.10) | *0.02* |
| Female donor | 2.11 (1.00-4.45) | *0.05* | 1.06 (0.54-2.08) | *0.86* |

°Time-dependent variable *Continuous variable [§]Defined as VGPR and CR

**TABLE 3**

**Cox models for overall and event free survivals**

| | Multivariable Analyses | | | |
|---|---|---|---|---|
| **Variable** | ***Overall survival*** | | ***Event free survival*** | |
| | **HR (95% CI)** | ***P-value*** | **HR (95% CI)** | ***P-value*** |
| Age* | 1.06 (1.00-1.14) | *0.05* | 1.07 (1.02-1.12) | *<0.01* |
| Ig-G Myeloma | 0.60 (0.26-1.34) | *0.21* | 0.52 (0.28-0.96) | *0.03* |
| International Staging System 3 | 1.91 (0.74-4.90) | *0.18* | 1.53 (0.73-3.20) | *0.26* |
| Disease in remission[§] at allografting | 0.20 (0.06-0.67) | *0.01* | 0.33 (0.17-0.65) | *<0.01* |
| HCT-Specific comorbidity Index ≥ 3 | 0.96 (0.27-3.36) | *0.95* | 0.87 (0.33-2.28) | *0.78* |

*Continuous variable [§]Defined as VGPR and CR

## FIGURE LEGENDS

***Figure 1*** Cumulative incidence estimates of graft-vs-host disease (GVHD) and mortality.

A. Acute GVHD: grade II-IV GVHD 38% (solid line); grade IV 3% (dotted line)

B. Transplant related mortality: 11%

C. Disease related mortality: 5.2% at 2 years; 20.5% at 5 years

***Figure 2*** Kaplan Meier estimates of overall and event free survivals after a follow up of 5 years: overall (A) and event free survivals (B) by intention to treat principle, overall (C) and event free survivals (D) among patients who completed program

***Figure 3***

Standard overall survival (gray solid line) by Kaplan-Meier, "current progression free survival" (dotted line) as described by Klein et al. (see text, **Methods section**) which includes responses to salvage therapies, and standard event free survival (black solid line) by Kaplan-Meier

***Figure 4***

**A.** Overall survival between patients without del13q (solid line; median not reached) and patients with del13q (dotted line; median 4.3 years)

**B.** Event free survival between patients without del13q (solid line; median 4.3 years) and patients with del13q (dotted line; median 2.2 years), P=0.01

**FIGURE 1**

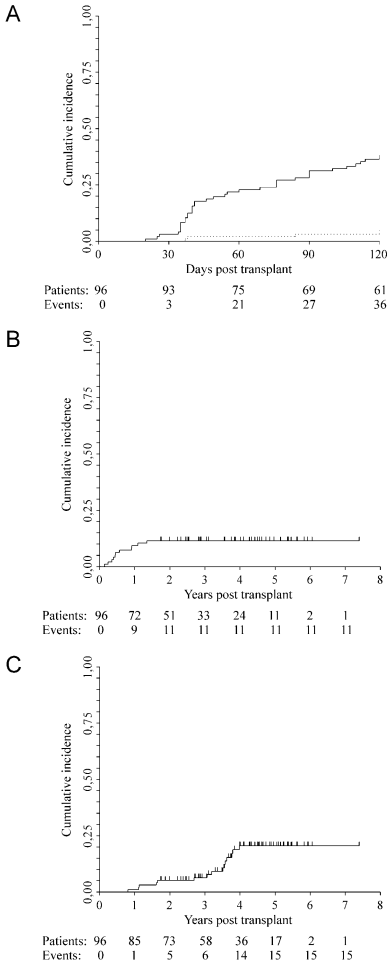

A
Cumulative incidence
0.00
0.25
0.50
0.75
1.00
0
30
60
90
120
Days post transplant
Patients: 96 93 75 69 61
Events: 0 3 21 27 36
B
Cumulative incidence
0.00
0.25
0.50
0.75
1.00
0
1
2
3
4
5
6
7
8
Years post transplant
Patients: 96 72 51 33 24 11 2 1
Events: 0 9 11 11 11 11 11 11
C
Cumulative incidence
0.00
0.25
0.50
0.75
1.00
0
1
2
3
4
5
6
7
8
Years post transplant
Patients: 96 85 73 58 36 17 2 1
Events: 0 1 5 6 14 15 15 15

**FIGURE 2**

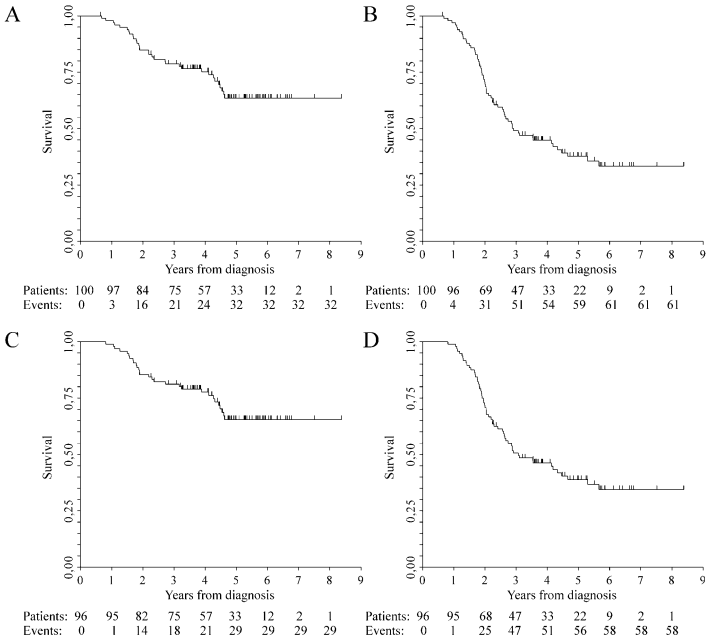

A
B
C
D
Survival
Years from diagnosis
Patients: 100 97 84 75 57 33 12 2 1
Events: 0 3 16 21 24 32 32 32 32
Patients: 100 96 69 47 33 22 9 2 1
Events: 0 4 31 51 54 59 61 61 61
Patients: 96 95 82 75 57 33 12 2 1
Events: 0 1 14 18 21 29 29 29 29
Patients: 96 95 68 47 33 22 9 2 1
Events: 0 1 25 47 51 56 58 58 58

**FIGURE 3**

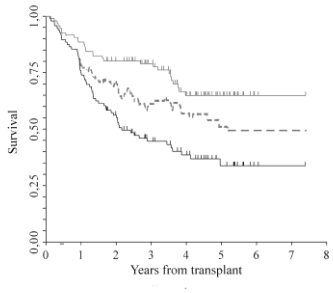
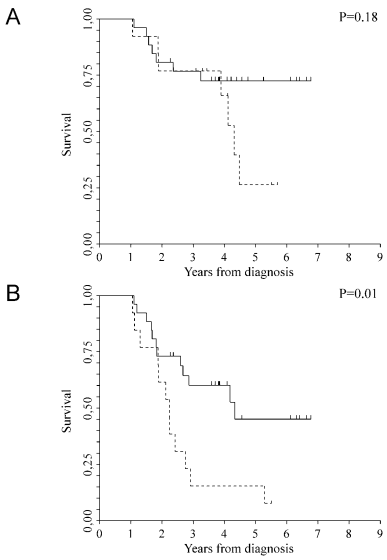

Survival
0.00
0.25
0.50
0.75
1.00
0
1
2
3
4
5
6
7
8
Years from transplant

**FIGURE 4**


A
P=0.18
Survival
0.00
0.25
0.50
0.75
1.00
0 1 2 3 4 5 6 7 8 9
Years from diagnosis
B
P=0.01
Survival
0.00
0.25
0.50
0.75
1.00
0 1 2 3 4 5 6 7 8 9
Years from diagnosis