Unrelated stem cell transplantation in multiple myeloma after a reduced intensity conditioning with pretransplant anti-thymocyte globulin is highly effective with low transplantation related mortality.

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Running-title: Dose-reduced conditioning and unrelated allografting for Multiple Myeloma

Key words: allogeneic stem cell transplantation, donor lymphocyte infusion, dose-reduced conditioning, non-myeloablative transplantation, graft versus host disease, unrelated donor, multiple myeloma

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Abstract:
We investigated the feasibility of unrelated stem cell transplantation in 21 patients with advanced stage II/III multiple myeloma after a reduced intensity conditioning regimen consisting of fludarabine (150 mg/m²), melphalan (100-140 mg/m²) and anti-thymocyte globulin (ATG: 3x10mg/kg). The median patient age was 50 years (range 32-61). All patients had received at least one prior autologous transplantation, in 9 cases as part of an autologous-allogeneic tandem protocol. No graft failure was observed. At day 40 complete donor chimerism was detected in all patients. Grade II-IV acute Graft versus host disease (GvHD) was seen in 8 (38%), while severe grade III/IV GvHD was observed in 4 patients (19%). Six patients (37%) developed chronic GvHD, but only two patients (12%) experienced extensive chronic GvHD. The estimated probability of non-relapse mortality at day 100 was 10% and at one year 26%. After allografting 40% of the patients achieved a complete remission, while 50% achieved a partial remission, resulting in an overall response rate of 90%. After a median follow-up of 13 months, the 2 years estimated overall and progression-free survival is 74% (95% CI: 54-94%) and 53% (95% CI: 29-87%), respectively. A shorter progression-free survival was seen in patients who already experienced relapse to prior autograft (26% vs 86%, p=0.04). Dose-reduced conditioning with pretransplant ATG followed by unrelated stem cell transplantation provides durable engraftment and donor chimerism, reduces substantially the risk of transplant related organ toxicity and induces high remission rates.

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Introduction:
Allogeneic stem cell transplantation from HLA-identical related donors resulted in 30% to 50% of the patients with multiple myeloma in long-term disease-free survival with well documented molecular remissions. There is a lower relapse rate in comparison to autologous transplantation which is probably due to a proven graft versus myeloma effect. Despite a recently better control of GvHD and of infectious complications, the most limiting factor for allogeneic stem cell transplantation is still the high transplant-related mortality, of 17 to 40%. Unrelated stem cell transplantation is increasingly being used in hematological disease such as CML with quite similar outcome to that of HLA-identical sibling donor transplantation. However, the limited experience with unrelated stem cell transplantation following standard conditioning regimens in multiple myeloma are discouraging. Recently, the NMDP reported a day 100 mortality of 41% in 71 myeloma patients who underwent allogeneic unrelated stem cell transplantation and the Seattle group reported a transplant-related mortality of 65% in 14 myeloma patients who were transplanted from unrelated or mismatched related donors. However, in the latter study two of the surviving patients are disease-free up to 7 years after transplantation. These results emphasize the urgent need for strategies to lower transplant-related mortality in myeloma patients after unrelated stem cell transplantation. So called non-myeloablative regimens based on fludarabine or low dose TBI demonstrated stable engraftment of allogeneic stem cells from related donors in patients with hematological diseases including multiple myeloma. However, in unrelated stem cell transplantation after a reduced intensity melphalan/fludarabine conditioning regimen the non-relapse mortality at day 100 was still 53% mainly due to severe GvHD. We report the results of a multicenter phase I/II study investigating the feasibility of a fludarabine-melphalan dose-reduced intensity regimen followed by unrelated stem cell transplantation in patients with advanced multiple myeloma. To lower the incidence of severe GvHD anti-thymocyte globulin, which has been used successfully in unrelated transplantation after standard conditioning regimens, was incorporated into the conditioning regimen.
Patients and Methods

Patient eligibility

Patients with advanced multiple myeloma were enrolled at 4 German and one Israeli transplant center. At each participating center the study was approved by the local Ethics Committee. A written informed consent was received from each patient. Patients with advanced multiple myeloma stage II/III, aged 18 to 65 with response or refractory to induction or salvage chemotherapy were eligible for the study protocol. Nine patients were treated in a sequential auto-allo tandem approach and 12 patients received an allografting after relapse or no change following an autologous transplant. To be included patients were required to have a sufficient cardiac function (ejection fraction >30%), a creatinine clearance greater than 30ml/h, a lung diffusion capacity of at least 50% and liver transaminases not greater than 3 times the upper limit of normal. HLA-A and -B antigens were typed by serologic methods, HLA-DRB1 and DQB1 alleles were typed with sequence-specific oligonucleotide probes. One antigen mismatch in class I or II was allowed. Unrelated donors gave informed consent according to the national protocol and stem cell mobilisation/collection or bone marrow harvesting was performed according to the accepted international and national standards and procedures.

Patient characteristics

Patient characteristics are shown in detail in table 1. Twenty-one patients were enrolled into the study between January 2000 and January 2002. Preliminary results of 7 patients who were treated in an autologous-allogeneic tandem protocol have been reported in a previous report and are now included with an extended follow-up. The median age of the patients was 50 years (range 32-61). There were 13 male and 8 female patients. Ten donors were male and 11 female. The median β2-microglobulin level at diagnosis was 2.9 mg/dl (range 1.0 to 7.0). Cytogenetic FISH analysis was available in 6 patients and showed a deletion 13 in one patient. Nine patients had received prior radiation therapy and the median number of previous chemotherapy cycles was 6 (range 4-15). All patients had received at least one previous cycle of high-dose chemotherapy followed by autologous stem cell transplantation. Seven patients had received two cycles of previous high-dose chemotherapy. Eleven patients experienced relapse after autologous transplantation, while 10 patients received allogeneic transplantation as consolidation therapy after an autograft, either as planned auto-allo protocol (n=9) or after no change to an autograft (n=1).
The disease status prior to allogeneic transplantation was CR (n=1), PR (n=8), MR (n=1), NC (n=3) and PD (n=8). Nineteen patients were matched for class I and II alleles, while two were mismatched in HLA-A and DRB1, respectively. The median time from diagnosis to transplant was 33 months (range 9 – 103). The serostatus of CMV of the recipient and the donor prior to transplantation was positive/positive in 9, positive/negative in 2, negative/negative in 8 and negative/positive in 2 cases. The source of stem cells was peripheral blood in 16 patients and bone marrow in 5 patients. No manipulation of the graft was performed.

Treatment plan
Treatment consisted of fludarabine 30 mg/m² intravenous infusion over 30 minutes on day –7 to –3 and melphalan intravenous infusion of 100 mg/m² on day –2. Anti-thymocyte-globulin (rabbit, Fresenius, Bad Homburg, Germany) was given at a dose of 10 mg/kg over 12 hours on day -3, -2 and –1, followed by allogeneic stem cell transplantation on day 0. Six patients received fludarabine 30 mg/m² only from day –5 to –3; Five patients received melphalan 140 mg/m². Because of two grade III acute GvHD the following patients (n=4) received ATG at a dose of 3 x 20 mg/kg. Granulocyte colony stimulating factor (G-CSF, 5 µg/kg) was given after allogeneic transplantation intravenously from day 5 and continued until sustained neutrophile engraftment. In case of relapse, incomplete chimerism or persistent disease on day 120 additional donor lymphocyte infusions were scheduled with an escalating regimen starting with 1 x10⁶ CD3+ cells/kg.

Supportive care
GvHD prophylaxis consisted of cyclosporine A (3 mg/kg, given from day –1 to day +100 after transplantation). The dose of cyclosporine A was adjusted to serum levels. Cyclosporine A was tapered from day 60 and discontinued at day 100 if no signs of GvHD were observed. Methotrexate (10 mg/m²) was given on day 1, 3 and 6 post transplantation. Two patients received mycophenolat mofetil (2 x1 gr from day +1 until day +28) instead of short course MTX. Acute GvHD was treated with high-dose steroids, and extensive chronic GvHD with cyclosporine A and steroids. All patients were nursed in single rooms with hepa-filtered air. Antibiotic prophylaxis consisted of ofloxacin or ciprofloxacin and antifungal prophylaxis of fluconazole and - in case of prior mycotic infection – of amphotericin B. Aciclovir was given as Herpes prophylaxis from day 1 until day 180. CMV seropositive patients received CMV-prophylaxis with ganciclovir from stable engraftment onwards until
day 100. Pneumocystis-carinii prophylaxis consisted of either trimethoprim and sulfamethoxazole on three days of the week or of a monthly inhalation with pentamidine. All blood products were irradiated before infusion and patients with seronegativity for CMV received only blood products from CMV-negative donors. Weekly monitoring of blood and urine for CMV-antigen by polymerase chain reaction (PCR) and short-term cultures of CMV lower matrix protein pp65-positive leukocytes were carried out. In case of positivity ganciclovir-treatment was initiated (5mg/kg of body weight intravenously, twice daily) and discontinued after negative test results were obtained. Regimen related toxicity was graded using the Bearman score. The maximum score for each organ system was recorded. Attempts were made to exclude toxicities due to GvHD from the therapy-related toxicity.

**Study objective**

The primary objective of the study was to assess engraftment, chimerism, acute graft versus host disease, toxicity, day 100 mortality and chronic GvHD in patients undergoing unrelated stem cell transplantation. Engraftment was defined as a leukocyte count of more than 1 x 10⁹/l for three consecutive days and an untransfused platelet count above 20 x 10⁹/l. Chimerism analysis was performed by allele-specific multiplex PCR technique. Genomic DNA was prepared from 200µl of blood and bone marrow using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. T-cells were obtained using CD2 MicroBeads and miniMACS columns (Miltenyi Biotec, Bergisch-Gladbach, Germany). A mixed chimerism was defined as the presence of at least 5% recipient DNA. The standard criteria were used for grading of acute and chronic GvHD. Chronic GvHD was evaluated in patients who survived at least 100 days with sustained engraftment. The secondary objective was to evaluate the response rate. Response to treatment was defined according to the EBMT/IBMTR criteria. Briefly, complete remission (CR) required a disappearance of monoclonal gammopathy in serum and urine as determined by immunofixation analysis for at least six weeks and less than 5% plasma cells in a bone marrow aspirate. A partial remission (PR) was defined as > 50% reduction and a minor response (MR) as >25% reduction of the paraprotein level, respectively. No change (NC) was defined as a less than 25% decrease or increase of the paraprotein. Relapse was defined as recurrence of the monoclonal protein or bone marrow plasmocytosis in case of prior CR.
Progression of non CR patients required at least a 25% increase of paraprotein or development of new bone lesions.

**Statistical Methods**

Survival curves for disease-free survival and overall survival were estimated by the Kaplan-Meier-method. The log rank test was performed for statistical analysis for time dependent analyses of survival, relapse and disease free survival. A p value of <0.05 was considered significant. Overall survival was calculated from transplantation until death from any cause. Progression-free survival was calculated from transplantation until progression or death from any cause.

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<th>Pat/Sex</th>
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<th>Prior high-dose chemotherapies</th>
<th>Time from Dx to Allo-Transplant (months)</th>
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<td>1</td>
<td>10</td>
<td>PD</td>
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</table>

Table1: Patient characteristics

Abbreviations: DX: diagnosis; CT: chemotherapy; m: male; f: female; CR: complete remission; PR: partial remission; MC: minor remission; NC: no change; PD: progressive disease.

**Results**

**Toxicity**

The major toxicity according to the Bearman scale was mucositis grade I in 24% and grade II in 64% of the patients. Liver toxicity grade I/II was observed in 35% of the patients and
resolved completely in all cases. No veno-occlusive disease was observed. Two patients developed grade II lung toxicity which resolved completely after steroid treatment. Because this lung toxicity might be attributed to fludarabine, the dose of fludarabine was reduced to 3 x 30mg/m² and subsequently no further lung toxicity was observed. Two renal failures were observed. In one patient renal function recovered completely and the other patient (no 21) with progressive Bence Jones proteinuria died from severe GvHD. One patient (no 7) developed fever and enlarged paraaortal lymphnodes. Due to a high EBV load as determined by PCR anti CD20 treatment was initiated. Two weeks after therapy he died on day 119 after unexpected sudden cardiac arrest in complete remission. No autopsy was performed. Positive CMV antigenemia by pp65 test was observed in three patients (14%), and two of them were treated successfully with ganciclovir. One patient with CMV seropositivity and negative donor showed repeated CMV antigenemia and was treated with combination of ganciclovir and foscavir. No CMV disease was noted. Two patients died of severe acute GvHD, one of these had Bence Jones proteinuria and renal failure; two patients of infections ( septicemia and cerebral aspergillosis). The estimated probability of non-relapse mortality was 10% at day 100 (95% CI: 1-19%) and 26% at 1 year (95% CI: 7-45%).

Engraftment

The median transplanted CD34+ cell number was 3.9 x 10⁶/kg BW (range 0.4 to 12.5 x 10⁶/kg BW). All patients became neutropenic (< 0.2 x 10⁹/L) and thrombocytopenic (< 20 x 10⁹/L) and required platelet and erythrocyte transfusions. No primary or secondary graft failure was observed. The median time until leukocyte (>1 x 10⁹/L) and platelet (>20 x 10⁹/L) engraftment was 16 (range 10-24) and 22 days (range 11-36), respectively.

Chimerism

Complete donor chimerism was detected in all patients by day 40. T-cell chimerism was evaluated in 11 patients and all showed complete donor chimerism on day 40 after allogeneic transplantation.

Graft versus Host Disease

Five patients (24%) did not show any signs of acute GvHD. Eight patients (38%) developed mild acute GvHD grade I, grade II was noted in 4 patients (19%). Grade II-IV acute GvHD was seen in 8 (38%), while severe grade III/IV GvHD was observed in 4 patients (19%). Two
cases of lethal grade IV GvHD were seen, one in a patient with HLA-mismatched
transplantation and the other in a patient with refractory disease.

Chronic GvHD was evaluated in 16 patient. 10 (63%) did not show any sign of chronic
GvHD. Six patients developed chronic GvHD (37%), 4 patients limited (25%) and only two
patients extensive (12%) cGvHD.

Response
After allogeneic transplantation 8 patients achieved a CR (40%) and 10 patients a PR (50%),
resulting in an overall response rate of 90%. Two of the patients with partial remission were
only positive in immunofixation and additional 4 patients show an ongoing decrease of
monoclonal bands. One patient (5%) achieved only a minor response and one patient
progressed after allogeneic transplantation (5%). 6 patients with PR prior to transplantation
converted to CR after allografting. From 8 patients with progressive disease prior to
transplantation, one converted to CR, 6 to PR and one remained progressive after unrelated
allografting (Table 2). So far, 4 patients have received donor lymphocyte infusions because of
progressive (n=3) or persistent disease (n=1). One patient developed grade II aGvHD and
showed a minor response, one patient neither sign of GvHD nor of tumor reduction. Two
patients are too early for evaluation.

Overall survival and disease-free survival

After a median follow-up of 386 days ( range 100 –769) the 2 years estimated overall survival
was 74% (95% CI: 54-94%) (Figure 1). In an univariate analysis there was a trend for better
overall survival for patients transplanted without relapse after autologous transplantation:
86% (95% CI: 63 - 99%) vs 64% (95% CI: 39 – 89%) p=0.3. Further univariate analnysis for
overall survival did not show significant difference for age (< or> 50years), β2microglobulin,
donor sex, remission status prior allograft (PR/CR vs NC/MR/PD), CMV-seropositivity, time
to transplant from diagnosis, stem cell source, acute and chronic GvHD and CD34 cell dose.
The 2 years estimated disease-free survival was 53% (95% CI: 29-87%). In an univariate
analysis only relapse after autologous transplantation resulted in a less favourable outcome in
comparison to patients without relapse: 26% (95% CI: 3 - 49%) vs 86% (95% CI: 63 - 99%)
(p=0.04) (Figure 2 and 3).
### Table 2: Outcomes

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<th>Engraftment Platelets#</th>
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<td>CR</td>
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<td>21. m</td>
<td>18</td>
<td>31</td>
<td>4</td>
<td>n.e.</td>
<td>PD</td>
<td>PD</td>
<td>Dead 64</td>
<td>GvHD, renal failure</td>
</tr>
</tbody>
</table>

**Abbreviations:** n.e.: not evaluable  
****: currently still decreasing monoclonal bands; * only positive for immunofixation  
** The patient had persistent disease on day 120 and received a donor lymphocyte infusion with now decreasing monoclonal bands  
CCR: continuous complete remission; PR: partial remission; PD: progressive disease; MR: minor remission; NC: no change; lim: limited chronic GvHD; ext: extensive chronic GvHD  
# days to absolute neutrophil count (ANC) >1 x 10^9/l, platelets >20 x 10^9/l  

Figure 1: Overall survival

![Overall survival graph](image-url)
Figure 2: Progression-free survival

![Graph showing progression-free survival over days after transplantation](image)

Figure 3: Progression-free survival in patients with and without relapse or progression after autologous transplantation

![Graph showing progression-free survival with relapse status](image)
Discussion

This study in patients with advanced multiple myeloma demonstrating that a dose-reduced conditioning regimen with allogeneic stem cell transplantation from unrelated donors is a feasible and highly effective treatment. The 2 years estimated overall survival of 74% and the disease-free survival of 53% is encouraging. However, it will require a longer follow-up to determine the curative potential of this approach for this otherwise fatal disease. Only few reports exist on the outcome of unrelated stem cell transplantation in multiple myeloma. In the largest report of the National Marrow Donor Program (NMDP) 71 patients received an unrelated stem cell transplantation after standard conditioning. A day 100 mortality of 41% and an estimated 5 years overall survival of only 17% were reported. Bensinger et al. reported a 64% treatment related mortality in 14 patients who were transplanted from unrelated or mismatched related donors. To reduce treatment related mortality after allogeneic stem cell transplantation, reduced intensity regimens based on TBI/fludarabin, busulfan/fludarabin or melphalan/fludarabin for related and unrelated stem cell transplantation have been developed. However, in unrelated stem cell transplantation GvHD and treatment related mortality remain a major problem. Giralt et al reported a day 100 mortality of 53% and a risk of acute GvHD grade II-IV of 62% after a melphalan/purine analog
containing reduced intensity regimen. Recently, Badros et al reported 6 patients with advanced multiple myeloma who were transplanted from unrelated donors after a reduced intensity regimen consisting of melphalan/fludarabin and low dose TBI (2.5 Gy). They reported a day 100 mortality of 33% and one primary graft failure was observed. In our study no graft failure occurred, only 38% of the patients developed acute stage II-IV GvHD and the estimated non-relapse mortality at day 100 was only 10%. One might ask why such differences occurred with nearly the same melphalan/fludarabin based regimen. The major difference to the studies mentioned above, is the incorporation of anti-thymocyte globulin into the conditioning regimen in our study. A similar incidence of acute grade II-IV GvHD (43%) was reported for dose-reduced conditioning followed by unrelated stem cell transplantation by Nagler et al, who also used ATG as part of the conditioning regimen consisting of busulfan and fludarabine. The ATG used in both studies was from rabbits (Fresenius-ATG). This in vivo depletion of activated T-cells in the recipient might facilitate engraftment, and due to the long half-life of the immunoglobulin an additional in vivo T-cell depletion of the graft might reduce the incidence of GvHD. Therefore, ATG as part of the conditioning regimen has been used successfully in unrelated stem cell transplantation to ensure engraftment and to reduce the incidence of severe GvHD. The major concern of any form of T-cell depletion is the high incidence of relapse. Attempts to overcome this high incidence of relapse by donor lymphocyte infusions were associated with a high incidence of acute and chronic GvHD. In contrast to other forms of T-cell depletion or other ATG preparations, the ATG used in our study does not lead to an significant increase of relapse in unrelated and related stem cell transplantation. Beside the anti-thymocyte globulin other forms of serotherapy have been described in allogeneic stem cell transplantation. Recently, stable engraftment after dose-reduced melphalan/fludarabin regimen incorporating pretransplant Campath-1H, a humanized anti-CD52 antibody have been described. In that study only 21% of the patients developed grade II-IV acute GvHD, but after donor lymphocyte infusion, which was given to 6 patients mainly to enhance remission status or donor chimerism 50% developed grade II/III acute GvHD.

Previous studies of allografting from related donors after standard conditioning clearly showed that early transplantation in the course of disease improves outcome. In our study we demonstrated that there was a significant better progression-free survival for those patients who were transplanted without prior failure to autologous transplantation. Other factors like age, time to transplant, response prior to transplant or β2 microglobulin did not significantly influence outcome, but this could be due to the limited number of patients and the short
We conclude that the melphalan/fludarabine reduced conditioning with pretransplant ATG followed by unrelated stem cell transplantation provides rapid and sustained engraftment with durable complete donor chimerism, and low day +100 treatment related mortality. Longer follow-up in a larger group of patients is needed to determine the late relapse and the curative potential. Because of the better outcome in patients without prior failure to autograft, allogeneic stem cell transplantation from unrelated donors should be investigated at an earlier phase of the disease.

References


Unrelated stem cell transplantation in multiple myeloma after a reduced intensity conditioning with pretransplant anti-thymocyte globulin is highly effective with low transplantation related mortality

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