treatment with or without radiotherapy provides a safe and effective option for patients with stage IE-IIE low-grade gastric MALT lymphomas. In terms of long-term relapse-free survival, our results compare very favorably with those from historical series in which antibiotic treatment was used as front-line therapy.[3-6] We think that the use of antibiotic therapy might be limited to particular situations (e.g., elderly patients, patients not eligible for surgery). In any case, surgery with or without radiotherapy remains the elective therapeutic procedure for patients who are refractory to, or who have relapsed after, antibiotic treatment.

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Allogeneic blood stem cell transplantation in advanced chronic myeloid leukemia - high response rate associated with increased chronic graft-versus-host disease

Twenty-four patients with advanced chronic myeloid leukemia in second chronic phase or in accelerated phase underwent allogeneic blood stem cell transplantation. After a median follow-up of 38 months (range: 4-69) 17 patients (70.8%) are alive, including 15 patients (62.5%) who are disease-free, whereas 4 patients (16.7%) have relapsed. Chronic graft-versus-host disease occurred in 13 of 21 (61.9%) patients at risk.

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Allogeneic stem cell transplantation remains the only curative option and induces long term molecular and cytogenetic remission in patients with advanced chronic myeloid leukemia (CML).[1] Single center series or international registries observed an overall survival of 20-25 % for patients transplanted in accelerated phase and a further decrease to about 10 to15 % for those transplanted in blast crisis.

Between 3/1997 and 8/2002 24 patients underwent peripheral blood stem cell transplantation (PBSCT) from either family or unrelated donors at our institution (Table 1). Accelerated phase and blast crisis were defined (by the presence of 10% to less than 20%, ≥ 20% blasts in marrow or peripheral blood, respectively) according to the WHO definition of CML.[2] The standard transplant-preparative regimen of 12 Gray (Gy) fractionated total body irradiation (TBI) in combination with a dose of 120mg/kg bodyweight of cyclophosphamide iv. was used in 18 patients (75%). Two patients (8.3%) received 8 Gy TBI and 120 mg/kg bodyweight cyclophosphamide, combined with a dose of 20 mg/kg anti-thymocyte globulin (Fresenius®) and 140 mg/m^2 fludarabine. One patient received 16 mg/kg bodyweight busulfan, 120 mg/kg cyclophosphamide and 200 mg/m^2 thiotepa. A reduced conditioning regimen with fludarabine (6×30 mg/m^2) busulfan (8 mg/kg bodyweight) and anti-thymocyte globulin (4×10 mg/kg bodyweight) was used in 3 patients (12.5%) due to prior radiotherapy or significant comorbidity.[3] All patients received 5 µg/kg granulocyte colony-stimulating factor (G-CSF) subcutaneously beginning on day +1 after PBSCT. Twenty patients (83.3%) received standard immunosuppression with cyclosporine 3 mg/kg bodyweight in combination with methotrexate on days 1, 3, 6 and 11. Seven patients who had matched unrelated donors or mismatched unrelated donors were additionally treated with 2 i.v. mycophenolate mofetil starting on day +10.

All patients showed leukocyte engraftment on a median of day +13.5 (range: 8-20) and 23 (95.6%) patients had platelet engraftment >20,000 ×10^9/L on a median of day +18 (range: 13-29). One patient died early on day +34 due to severe graft-versus-host disease (GvHD) and ongoing pancytopenia after initial myeloid engraftment. Acute GvHD ≥ was observed in 12 patients (50%) with 3 patients (12.5%) having grade III or IV. Limited and extensive chronic GvHD occurred in 6 (28.6%) patients each. Three patients (14.3%) developed *de novo* limit-

Table 1. Characteristics of patients and grafts.

Number of patients	24
Gender (female/male)	11/13
Median age in years (range)	39.1 (20-52)
Median time from diagnosis to transplantation in months (range)	29 (4-76)
Median number of previous therapies	2.5 (1-4)
Seropositivity of recipient and/or donor for cytomegalovirus	16 (67%)
Disease status at diagnosis	
chronic phase	13 (54.2%)
accelerated phase	8 (33.3%)
blast crisis	3 (12.5%)
Disease status at transplantation	
accelerated phase	11 (45.8%)
accelerated phase after blast crisis	5 (20.8%)
second chronic phase	8 (33.3%)
CD34+ cells×10^6 per kg bodyweight (range)	7.13 (2.31-19.0)
CD3+ cells×10^6 per kg bodyweight (range)	312.8 (163.6-793.4)
Fully-matched HLA family donors	8 (33.3%)
Mismatched HLA family donors	6 (25%)
Matched unrelated donors	8 (33.3%)
Mismatched unrelated donors	2 (8.3%)

ed (n=2) or extensive (n=1) chronic GvHD. Nine chronic GvHD patients were still on immunosuppressive medication.

Complete cytogenetic and molecular remissions (BCR/ABL transcripts by molecular analysis fluorescence *in situ* hybridization and polymerase chain reaction) of CML were achieved in 20 (83.3 %) of 22 evaluable patients at day 60-120 after PBSCT. Three (12.5%) patients died after 32, 61 and 65 days, due to sepsis and graft-failure (n=1), subdural bleeding and sepsis (n=1), and grade III GvHD with therapy-refractory thrombotic-thrombolytic microangiopathy (n=1), respectively. Three patients with extensive chronic GvHD disease died due to CMV pneumonia (11 months), severe thrombotic-thrombolytic microangiopathy (7 months), and bronchiolitis obliterans (23 months). Four patients (day +46, +79, +169, +176 after transplant) relapsed, resulting in a relapse rate of 16.7%. Two of them died, whereas the two others are currently again in a complete hematologic and molecular remission, induced with imatinib and donor lymphocyte infusions, modulating immunosuppressive medication. Risk-factors for event-free survival in univariate analysis showed an event-free survival of 80-87.5 % for age < 40 years and 2nd chronic phase at transplant versus 50% for >40 years and accelerate phase.

The overall survival of 70% after a median follow-up of three years (Figure 1) confirms data from a retrospective register,[4] in which event-free-survival was greater in patients with advanced CML after transplantation with PBSCT than in patients after bone marrow transplantation.

In a population of patients with advanced disease, the low therapy-related mortality, less than 10%, in the first 100 days

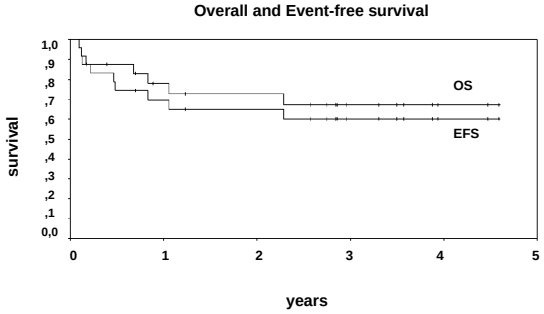

Figure 1. Overall survival (OS) and Event-free-survival (EFS) in advanced CML after allogeneic blood stem cell transplantation (n = 24), Kaplan-Meier curve

after transplant, was notable. For adult CML patients, the observed incidence of grade II-IV acute GvHD of 50 % was acceptable, especially with regard to the spectrum of available donors. In a meta-analysis of acute and chronic GvHD after bone marrow transplantation and PBSCT the risk of disease relapse was lower after PBSCT, although this decrease did not reach statistical significance.[5] However, in these reports[6-8] no stratification for chronic leukemia versus acute leukemia was performed for the GvHD analysis. Allogeneic stem cell transplantation with peripheral blood stem cells is highly effective in advanced CML when a second chronic phase is induced prior to transplantation. Our coincidental finding of a high incidence of chronic graft-versus-host disease associated with the duration of event-free survival seems to reflect an enhanced graft-versus-leukemia effect. The high rate of chronic GvHD is associated with morbidity and mortality from non-leukemia death.

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Analysis of immune reconstitution in adults undergoing non-myeloablative allogeneic peripheral blood stem cell transplantation

The effect of non-myeloablative procedures on post-transplant immune reconstitution is unknown. We investigated the immune status of patients with leukemia following non-myeloablative allogeneic peripheral blood stem cell transplantation (NST). Ten adult were analyzed 1, 3 and 12 months after transplant. We conclude that NST may result in early immune reconstitution.

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Immune reconstitution plays a pivotal role in the long-term outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT), not only because immune defects are related to infectious morbidity post-transplant, but also because they may influence the risk of relapse and the development of secondary malignancies after HSCT.[1] Following conventional allo-HSCT, all patients experience a period of profound neutropenia and immunodeficiency that is significantly responsible for the serious infectious complications that can occur after a transplant. The entire strategy of non-myeloablative preparative regimen relies on the graft-versus-leukemia effect or the graft-versus-tumor effect as the primary therapeutic modality. Non-myeloablative stem cell transplantation (NST) has reduced conditioning-related toxicity,[2] but the effects on post-transplant immune recovery have not been studied in detail. We evaluated several immunologic parameters of patients who underwent NST at our institution.

Ten patients who had undergone NST from HLA-identical siblings were analyzed as a case group. The conditioning regimen consisted of fludarabine (Schering AG, Berlin, Germany) 30 mg/m^2/day for 5 days, busulfan 2 mg/kg/day for 4 days and anti-T lymphocyte globulin (ATG, Fresenius AG, Munich, Germany) 10 mg/kg/day for 5 days. Allogeneic hematopoietic blood stem cells were collected following mobilization with granulocyte colony-stimulating factor (G-CSF) (Kirin-Sankyo, Tokyo, Japan) 10 μg/kg/days for 5~7 days. G-CSF-mobilized blood stem cells were used with no further *in vitro* manipulation. The CD34$^+$cell count was 5×10^6/kg. Prophylaxis against GVHD included cyclosporine A 2 mg/kg/day starting on day –1 and continued at a dose to maintain therapeutic blood levels until day +100. Patients received a donor lymphocyte infusion (DLI) of 1×10^7 CD3$^+$cells/kg on day +30. DLI were given in graded incremental doses. Two- or three-color flow cytometry of CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, CD3$^-$/CD16$^+$/CD56$^+$, CD3$^+$CD25$^+$ cell surface markers was performed 1, 3 and 12 months after NST. White blood cell counts were assessed at each point of blood collection using an automatic cell counter. T-cell proliferative responses, LAK and NK activity, and serum immunoglobulin (Ig) were examined according to the previous report.[3,4] Donor-recipient chimerism was assessed by polymerase chain reaction (PCR)-based amplification of a polymorphic short tandem repeat (STR) region.

The absolute number of CD3$^+$ and CD8$^+$ T cells, and B lymphocytes, as well as the proliferative response to T-cell mitogens, recovered with time after transplantation. CD8$^+$ T cells and B cells recovered to the normal range by 3 months. CD4$^+$ T-cell counts remained below normal up to 1 year after transplantation. Recovery of NK cell number and innate cytotoxic activity was fast. IgG and IgM levels were within normal range by 1 month post-transplant (Table 2) .

Little is known about immune reconstitution following non-myeloablative allogeneic transplantation. Immune reconstitution in this particular setting depends on three potential sources of functional lymphocytes; (i) residual host lymphocytes that survived after the conditioning regimen; (ii) naive, stem cell-derived lymphocytes of both donor and host origin; and; (iii) mature donor lymphocytes transfused as part of our transplant protocol. Our analysis is in accordance with data published previously.[4-6] The different behavior in the immune reconstitution of the CD8$^+$ subset after NST may be favored by an extrathymic origin of these cells while CD4$^+$ subset recovery, which is thymus-dependent, is impaired because of thymic involution.[7] The proliferative response of T cells to polyclonal activators (PHA) was high, which contrasts with the impaired immune reactivity observed in patients conditioned by a conventional myeloablative regimen.[8] Therefore, we conclude that, following a non-myeloablative regimen, patients may conserve an almost intact *in vitro* T cell-dependent proliferative response.

All transplanted patients investigated in the present study displayed normal or high levels of LAK and NK activity, especially during the early period post-transplantation. It is important to establish that non-myeloablative regimens do not suppress NK cell activity since NK cells may play a role in engraftment, in prevention of GVHD and in exerting graft-versus-leukemia effects.[9]

A two-step strategy has been developed to reduce the toxicity of conditioning regimens and to preserve a curative antitumoral effect corresponding to that of allo-HSCT after a non-myeloablative preparative regimen, whether followed by DLI or not, as documented by the results of both chimerism and minimal residual disease studies. The DLI contributed mostly mature T cells, which may contribute to improved T-cell function following NST. Bellucci *et al.*[10] demonstrated that DLI developed increased numbers of B cells. Further studies in animal model systems as well as in patients who receive DLI will be necessary to define the mechanism underlying this immunologic effect better.

Large cohorts of patients must be investigated to determine