



Conditioning regimens

Comparison of total body irradiation vs busulfan in combination with cyclophosphamide as conditioning for unrelated stem cell transplantation in CML patients

N Kröger¹, T Zabelina², W Krüger¹, H Renges¹, N Stute¹, H Kabisch¹, N Jaburg¹, C Löliger³,
A Krüll⁴ and AR Zander¹

¹Bone Marrow Transplantation, ³Department of Transfusion Medicine, and ⁴Department of Radiation Therapy, University-Hospital Hamburg, Germany; and ²Pavlov University, St Petersburg, Russia

Summary:

We compared fractionated total body irradiation (12 Gy)/cyclophosphamide (120 mg/kg) with busulfan (16 mg/kg)/cyclophosphamide (120 mg/kg) as preparative therapy in unrelated donor stem cell transplantation of CML patients. Fifty patients with CML (1.CP = 46; aP = 4) and a median age of 36 years (range 16–52) were enrolled in this sequential trial between 1994 and 1999. In both groups patients were well balanced with respect to age, disease status, stem cell source and CMV status. All patients received standard doses of cyclosporin A, methotrexate and anti-thymocyte globulin (ATG) as GVHD prophylaxis. No graft failures occurred in either group. The median day of leukocyte engraftment was earlier in the Bu/Cy than in the TBI/Cy group (day 15 vs 17; $P = 0.006$). The incidence of grade II–IV GVHD was 40% in the TBI/Cy and 36% in the Bu/Cy group, whereas severe grade III/IV GVHD was only observed in 12% of patients in both groups. The incidence of chronic GVHD (limited and extensive) at 1 year was higher in the Bu/Cy arm (65% vs 30%; $P = 0.02$). More toxicity grade I/II of the liver (88% vs 44%; $P = 0.002$) and more hemorrhagic cystitis (32% vs 8%; $P = 0.02$) were observed in the Bu/Cy regimen. Seven relapses in the TBI and no relapse in the Bu/Cy group were observed after a median follow-up of 44 and 15 months, respectively. The estimated 3 year OS and DFS was 72% (95% CI: 55–98%) and 58% (95% CI: 39–77%) in the TBI and 70% (95% CI: 51–89%) for DFS and OS in the Bu/Cy group. We conclude that the anti-leukemic effect of the Bu/Cy regimen seems to be at least as effective as the TBI/Cy combination in unrelated stem cell transplantation of CML patients, with no graft failures, but that it correlates with a higher incidence of liver toxicity, hemorrhagic cystitis and chronic GVHD. Longer follow-up is necessary to determine the late

relapse rate and late toxicity. *Bone Marrow Transplantation* (2001) 27, 349–354.

Keywords: anti-thymocyte globulin; unrelated bone marrow transplantation; total body irradiation; busulfan; chronic myeloid leukemia

Allogeneic bone marrow transplantation is the only proven curative therapy for patients with chronic myeloid leukemia. Only about 30% of candidates for allogeneic bone marrow transplantation have an HLA-identical sibling. Therefore, bone marrow transplantation from unrelated HLA compatible donors has become an alternative option and is now considered standard therapy for young patients with CML lacking a suitable sibling donor. However, the results of unrelated bone marrow transplantation compared to those from sibling donors seem to be inferior, because of higher morbidity and mortality due to graft failure, infections and graft-versus-host disease (GVHD).^{1,2} Total body irradiation (TBI) in combination with cyclophosphamide is the most frequently used preparative regimen in unrelated bone marrow transplantation of CML.^{3–5} The radiation-free regimen of busulfan and cyclophosphamide has been proven effective for eradication of leukemia and sufficiently immunosuppressive for engraftment in HLA-matched sibling transplantation. In randomized trials of Bu/Cy vs TBI/Cy in HLA-matched sibling transplantation of CML patients, the combination of busulfan/cyclophosphamide was shown to be at least as effective as TBI/cyclophosphamide.^{6,7} Furthermore, the French study⁶ reported a higher antileukemic effect and the Seattle group⁷ a significantly lower toxicity of the busulfan/cyclophosphamide combination. In unrelated bone marrow transplantation the TBI regimen is commonly used because TBI might be required to obtain the degree of immunosuppression needed for engraftment. In several single center reports a 0% to 60% incidence of graft failure in unrelated bone marrow transplantation was observed using the busulfan/cyclophosphamide combination as preparation.^{8–11} In the present study we report the results of 50 CML patients who underwent allogeneic unrelated stem cell transplantation between 1994 and 1999 at our center. In

this sequential trial patients transplanted before 1996 were treated with TBI/cyclophosphamide, whereas subsequent patients received busulfan/cyclophosphamide as conditioning.

Materials and methods

Patient population

The study group consisted of 50 patients with chronic myeloid leukemia receiving unrelated donor stem cell transplants between 1994 and 1999 at the University Hospital Eppendorf in Hamburg. Written informed consent was obtained from each patient, and the study was approved by the local Ethics Committee. Both groups were well balanced with respect to age, CMV status, stem cell source and disease status. Patient characteristics are shown in Table 1.

HLA-typing and donor matching

HLA-A and -B antigens were typed by serological methods, HLA-DRB1 alleles were typed with sequence-specific oligonucleotide probes. In all patients, a pretransplantation lymphocyte cross match with patient sera and donor cells was performed. There were 43 patients completely matched

for HLA-B and -DRB1 and seven patients were mismatched either at HLA-B or -DRB1.

Transplant preparative regimen

Twenty-five patients received conditioning with total body irradiation, 1200 cGy given over 3 days in six fractions, and 25 patients received busulfan (14 to 16 mg/kg), both followed by cyclophosphamide (120 mg/kg). Busulfan was administered orally in four divided doses daily for 4 days (day -8 to -5) and cyclophosphamide 60 mg/kg was given intravenously over 1 h for 2 days (day -4 and -3). Phenytoin was given to prevent busulfan-induced seizures for another 2 days after stopping busulfan. Uroepithelial prophylaxis was achieved with hyperhydration and mesna. Bone marrow or peripheral blood stem cells were infused on day 0. The stem cell source was bone marrow in 44 patients, and peripheral blood stem cells in six patients. No manipulation of the graft was performed.

GVHD prophylaxis

GVHD prophylaxis consisted of cyclosporin A (3 mg/kg, given from day -1 to 6 months post transplantation). The dose of cyclosporin A was adjusted to a serum level of

Table 1 Patient characteristics

Variable	TBI/Cy n = 25	Bu/Cy n = 25	P value
Median age (range)	36 (21–51)	26 (16–52)	NS
Median age of donor (range)	32 (19–50)	34 (23–57)	NS
Gender patients: M:F	14:11	15:10	NS
Gender donors: M:F	17:8	14:11	NS
BMT <2 years from diagnosis	15	19	NS
BMT >2 years from diagnosis	10	6	NS
Disease-status:			
1. CP	22	24	
Accelerated phase	3	1	NS
Sex donor/recipient: matched/mismatched	18:7	15:10	NS
HLA			
Matched	24	19	NS
Mismatched	1	6	0.04
DRB1	1	3	
HLA-B	0	3	
ABO			
Identical	7	5	NS
Non-identical	17	20	NS
Stem cell source			
BM	23	21	NS
PBSC	2	4	NS
ATG-dose			
90 mg/kg	25	18	
60 mg/kg	–	1	
30 mg/kg	–	5	
20 mg/kg	–	1	
Median CD34 ⁺ cells × 10 ⁶ /kg (range)	3.8 (0.6–17) ^a	3.7 (1.4–10) ^b	NS
Median MNC × 10 ⁸ /kg (range)	1.3 (0.4–11)	1.3 (0.4–14)	NS
CMV-status			
Positive (recipient and/or donor)	14	15	NS
Negative (recipient and donor)	11	10	NS

^an = 20.

^bn = 24.

200–300 ng/ml. Cyclosporin A was tapered from day 84 and discontinued at day 180. Methotrexate (10 mg/m²) was given on day 1, 3 and 6 post transplantation. Anti-thymocyte globulin (rabbit, Fresenius, Bad Homburg, Germany) was given to all patients; 43 patients received a dose of 30 mg/kg over 12 h on days –3, –2 and –1, and seven patients received a cumulative dose of ATG between 20 and 60 mg/kg. Patients received intravenous globulin on day 1, 3, 7, 14, 21, 28, 56, 84 and day 120. To prevent gut GVHD metronidazole (Clont, Fa Bayer, Leverkusen, Germany) at a dose of 500 mg i.v., was given 3 times a day from conditioning until discharge. The standard criteria were used for grading acute and chronic GVHD.¹² Acute GVHD was treated with high-dose steroids, and extensive chronic GVHD with cyclosporin A and steroids. Chronic GVHD was evaluated in patients who survived at least 80 days with sustained engraftment.

Regimen-related toxicity

Regimen-related toxicity affecting the renal, hepatic, cardiac, pulmonary, gastrointestinal, CNS system and mucous membranes was graded using the Bearman score.¹³ The maximum score for each organ system was recorded. Attempts were made to exclude toxicities due to GVHD from therapy-related toxicity. Veno-occlusive disease of the liver was graded according the Seattle criteria.¹⁴

Supportive care

All patients were nursed in single rooms with HEPA-filtered air. Antibiotic prophylaxis consisted of ofloxacin or ciprofloxacin, and antifungal prophylaxis was fluconazole, and – in case of prior mycotic infection – amphotericin. Aciclovir was given as herpes virus prophylaxis from day 1 until day 180. *Pneumocystis carinii* prophylaxis consisted of either trimethoprim and sulfamethoxazole or monthly inhalation of pentamidine for 1 year. All blood products were irradiated before infusion, and only blood products from CMV-negative donors were given.

Weekly monitoring of blood and urine for CMV-antigen and by PCR and short-term culture was carried out. In cases of repeated positivity ganciclovir treatment was initiated.

All patients received hematopoietic growth factors (G-CSF) intravenously beginning on day 1 and continued until the absolute granulocyte count was >1.0 /nl for 3 consecutive days. Prostaglandin E1 (Prostavasine, Fa Schwarz-Pharma, Mannheim, Germany) at a dose of 500 µg was given daily as a continuous infusion for patients with hepatotoxicity upon a rise of total bilirubin above 2.0.

Statistical methods

Statistical analysis was performed by using WIN-STAT-software (Kalmia, Cambridge, MA, USA). GVHD and survival were analyzed by life table methods according to the Kaplan–Meier method. Differences in survival and GVHD were studied using the log-rank test. For comparison, the independent *t*-test was used. A *P* value of <0.05 was considered significant.

Results

Engraftment

No graft failures were observed in either group. The median number of transplanted CD34⁺ cells/kg was well balanced between the two groups: 3.8 × 10⁶/kg in the TBI group and 3.7 × 10⁶/kg in the Bu/Cy group (*P* = 0.4). However, leukocyte engraftment (>1000/mm³) was faster in the Bu/Cy group (median 15 (range: 11–20) vs 17 days (range 12–24)) (*P* = 0.006). Platelet engraftment (>20000/mm³) was reached for Bu/Cy and for TBI/Cy after a median of 21 and 24 days, respectively (NS *P* = 0.2).

Graft-versus-host disease

Nine patients (36%) in the TBI/Cy group and 11 patients (44%) in the Bu/Cy group experienced no signs of acute GVHD. Thirteen patients (52%) in the TBI group and 11 (44%) patients in the Bu/Cy group developed mild acute GVHD grade I or II. Severe grade III/IV GVHD occurred in only three patients in each group (12%). Chronic GVHD was observed in six patients in the TBI/Cy and 11 patients in the Bu/Cy group, resulting in a probability of chronic GVHD at 1 year of 65% in the Bu/Cy and 30% in the TBI/Cy group (*P* = 0.02). Extensive chronic GVHD was more common in the Bu/Cy arm (6 vs 3 patients), even if patients with HLA-mismatched donors were censored.

Toxicity and treatment-related mortality

Toxicity of the liver grade I or II according to the Bearman scale was higher in the Bu/Cy than in the TBI group (88% vs 44%; *P* = 0.002). Additionally, a trend for more moderate VOD was observed in the Bu/Cy group (56 vs 32%; *P* = 0.1). Hemorrhagic cystitis was seen more often in the Bu/Cy arm (32% vs 8%, *P* = 0.02). Other toxicities such as mucositis or renal toxicity were observed to the same extent in both groups (Table 2). Treatment-related mortality (TRM) was slightly higher in the Bu/Cy than in the TBI group (28% vs 20%; *P* = 0.6) and was mainly due to interstitial pneumonia, GVHD and infections (see Table 3).

Relapse

Relapse was defined as hematologic relapse. After a median follow-up of 34 months (range 5–65), seven relapses (14%) were observed after a median of 12 months post transplantation (range, 3–50). All relapses occurred in the TBI group, but the median follow-up of the TBI group is longer than is that of the Bu/Cy group (44 vs 15 months). All patients with hematologic relapses received donor lymphocyte infusion. Three patients did not respond to DLI and two of these received a second transplant and are free of disease at the time of this evaluation. The third patient developed blast crisis and expired. Two patients died of severe GVHD and sepsis, respectively. Two patients responded to DLI with complete disappearance of the bcr/abl transcript.

Table 2 Results

Variable	TBI/Cy n = 25	Bu/Cy n = 25	P value
Acute GVHD			
Grade II–IV	40%	36%	NS
Grade III/IV	12%	12%	NS
Chronic GVHD overall (at 1 year)	30%	65%	0.02
Limited	17%	48%	NS
Extensive	16%	33%	NS
Engraftment: median days (range)			
Leukocyte >1000/mm ³	17 (12–24)	15 (11–20)	0.006
Platelets: >20000/mm ³	24 (15–43) ^a	22 (13–41) ^b	NS
Toxicity: (Bearman)			
Hepatic I/II	60%	88%	0.002
Renal I/II	35%	44%	NS
Mucositis I/II	96%	96%	NS
Hemorrhagic cystitis	8%	32%	0.02
Veno-occlusive disease			
Mild	1	1	NS
Moderate	8	14	NS
TRM	20%	28%	NS
3 years estimated OS	72%	70%	NS
3 years estimated DFS	58%	72%	NS

^an = 17.

^bn = 22.

^cFollow-up TBI/Cy = 44 months; Bu/Cy = 15 months.

Table 3 Treatment-related mortality

TRM	TBI/Cy	Bu/Cy
Interst. pneumonia	0	2
GVHD	1	1
CMV disease	1	2
Sepsis/bleeding	1	1
Aspergillus	2	1
Overall	5 (20%)	7 (28%)

Overall and disease-free survival

After a median follow-up of 44 months (range, 34–65) in the TBI and of 15 months (range, 4–34) in the Bu/Cy group, the 3-year estimated overall survival (OS) is 72% (95% CI: 55–98%) for the TBI group and 70% (95% CI: 52–98%) for the Bu/Cy group (*P* = 0.7). The 3-year estimated disease-free survival (DFS) is 58% (95% CI: 39–77%) for the TBI and 70% (95% CI: 51–89%) for the BU/Cy regimen (*P* = 0.7) (Figures 1 and 2). Age (< or >40 years), incidence of acute or chronic GVHD or HLA-matched/mismatched donor did not affect outcome (data not shown). Patients transplanted with a CD34⁺ cell dose above 3.0 × 10⁶/kg had a better 3-year estimated overall survival than did patients transplanted with a CD34⁺ cell dose below 3.0 × 10⁶/kg (71 vs 50%; *P* = 0.05).

Discussion

This is the first study comparing TBI with busulfan combined with cyclophosphamide as a preparative regimen in CML patients transplanted from an unrelated donor.

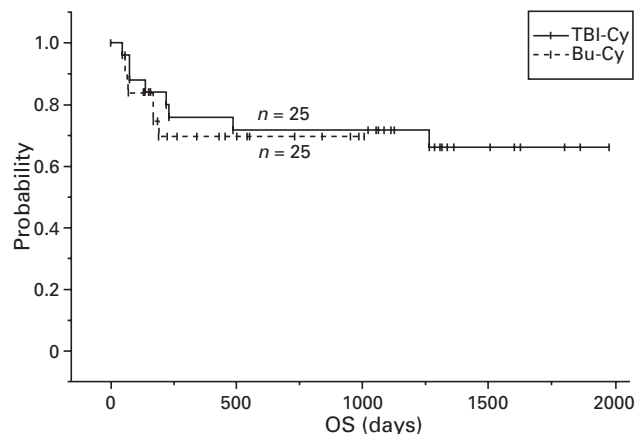


Figure 1 Overall survival of CML and unrelated stem cell transplantation after conditioning with TBI/Cy or Bu/Cy.

TBI/cyclophosphamide is the most commonly used regimen in unrelated stem cell transplantation in patients with CML, because TBI may be necessary to achieve the degree of immunosuppression needed for engraftment since it has been that shown unrelated bone marrow transplantation is associated with a higher incidence of graft failure.^{2,15} The combination of busulfan and cyclophosphamide was first described by Santos *et al.*¹⁶ Busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg) provided an effective alternative to TBI plus cyclophosphamide in treatment of AML. Modification of this regimen by lowering the cyclophosphamide dose to 120 mg/kg appears to be equally effective and less toxic.¹⁷ In an earlier study, the Bu/Cy regimen in unrelated bone marrow transplantation appeared to be associated with a higher incidence of graft failure than that reported after conditioning with TBI.⁸ Recently, several single center

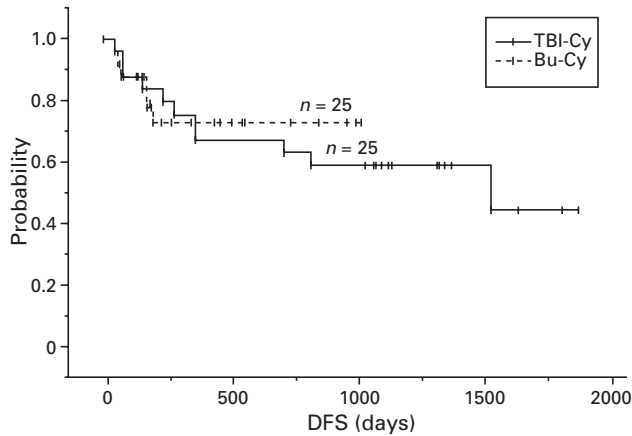


Figure 2 Disease-free survival of CML and unrelated stem cell transplantation after conditioning with TBI/Cy or Bu/Cy.

reports have demonstrated a low incidence of graft failure following conditioning with busulfan and cyclophosphamide.^{9–11,18} In our study all patients, including the six transplanted with one HLA-mismatch, conditioned with the busulfan/cyclophosphamide regimen had rapid and sustained engraftment. There are two randomized trials of Bu/Cy vs TBI/Cy in related bone marrow transplantation for CML. The French Society of Bone Marrow Grafting found no significant differences with respect to overall and disease-free survival between the TBI/Cy and the Bu/Cy regimen, but they reported a higher incidence of graft failure for the Bu/Cy regimen and an increased risk of relapse after the TBI/Cy regimen, especially when fractionated TBI was given.⁶ The other randomized trial from Seattle also reported no differences in disease-free and overall survival between the Bu/Cy and the TBI/Cy regimens, but the Bu/Cy regimen was better tolerated than was the TBI/Cy combination.⁷ In a third retrospective study from the Nordic Bone Marrow Transplantation Group of TBI/Cy vs Bu/Cy in leukemia patients, a higher incidence of VOD (12% vs 1%) and hemorrhagic cystitis (24% vs 8%) was observed in the Bu/Cy arm. In the subgroup of CML patients no difference in overall and disease-free survival was seen.¹⁹ In our study, the relapse-rate was higher in the TBI/Cy group similar to the observation in the French study for related bone marrow transplantation. However, no definite conclusions should be drawn from this preliminary observation because of the different follow-ups in each arm (TBI/Cy 44 months and Bu/Cy 15 months). At time of this analysis, we found no differences in the 3-year estimated OS (72% vs 70%) and the DFS (58% vs 70%) between the TBI and the Bu/Cy group. We observed a trend for higher TRM in the Bu/Cy group (28 vs 20%) and more liver toxicity with Bu/Cy ($P = 0.002$). We further noted a higher incidence of hemorrhagic cystitis in the Bu/Cy group (32% vs 8%), as was observed by the Nordic Bone Marrow Transplantation Group. Because of the oral formulation of busulfan and the differences in resorption, i.v. preparations of busulfan will facilitate its use, with more predictable serum levels and hence lower toxicity.²⁰ Chronic GVHD was more frequently seen in the Bu/Cy group ($P = 0.02$). HLA-mismatch and PBSC as the stem cell source are

known to be associated with a higher incidence of chronic GVHD, but even if these patients were censored, the incidence of cGVHD was twice as high in the Bu/Cy group (8 vs 4 patients). This is in accordance with the findings of the Nordic Bone Marrow Transplantation Group,¹⁹ who also observed a higher incidence of cGVHD in related bone marrow transplantation after Bu/Cy than after TBI/Cy (45% vs 35%, $P = 0.04$). The reasons for this observation are not clear, but the higher toxicity of the Bu/Cy regimen might have contributed to the higher incidence of cGVHD.

The outcome of unrelated bone marrow transplantation in CML has been adversely affected by age,^{2,4,21} HLA-disparity, incidence of GVHD and by the interval from diagnosis and transplantation.^{4,22} In our study, neither age (< or >40 years), incidence of acute or chronic GVHD or an HLA-matched/mismatched donor affected outcome. However, the cell dose transplanted did affect overall survival, but not disease-free survival, which confirmed the importance of cell dose recently reported by several investigators.²³ The low incidence of severe GVHD and absence of graft failure in the entire study might be due to the incorporation of anti-thymocyte globulin in the preparative regimen, which has been shown to be effective in preventing severe GVHD and graft failure in HLA-matched and mismatched unrelated stem cell transplantation.^{24–26}

In conclusion, our preliminary results demonstrate that the anti-leukemic effect of the Bu/Cy regimen seems to be at least as effective as the TBI/Cy combination in unrelated stem cell transplantation of CML patients without graft failure, but with a higher incidence of liver toxicity, hemorrhagic cystitis and chronic GVHD. Longer follow-up is necessary to determine late relapse rate and toxicity.

Acknowledgements

We thank the staff of the BMT unit for providing excellent care of our patients and the medical technicians for their excellent work in the BMT laboratory.

References

- 1 Marks D, Cullis J, Ward K *et al*. Allogeneic bone marrow transplantation for chronic myeloid leukemia using sibling and volunteer unrelated donors: a comparison of complications in the first 2 years. *Ann Intern Med* 1993; **19**: 207–214.
- 2 McGlave P, Bartsch G, Anasetti C *et al*. Unrelated donor marrow transplantation for chronic myelogenous leukemia: Initial experience of the National Marrow Donor Program. *Blood* 1993; **81**: 543–550.
- 3 Kernan NA, Bartsch G, Ash RC *et al*. Analysis of 462 transplantations from unrelated donors facilitated by the national marrow donor program. *New Engl J Med* 1993; **328**: 593–602.
- 4 Hansen JA, Gooley TA, Martin PJ *et al*. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. *New Engl J Med* 1998; **338**: 962–968.
- 5 Spencer A, Szydla RM, Brookes PA *et al*. Bone marrow transplantation for chronic myeloid leukemia with volunteer unrelated donors using *ex vivo* or *in vivo* T-cell depletion: major prognostic impact of HLA class I identity between donor and recipient. *Blood* 1995; **86**: 3590–3597.
- 6 Devergie A, Blaise D, Attal M *et al*. Allogeneic bone marrow

- transplantation for chronic myeloid leukemia in first chronic phase: a randomized trial of busulfan-cytosin versus cytosin-Total-body irradiation as preparative regimen: a report from the French Society of Bone Marrow Graft. *Blood* 1995; **85**: 2263–2268.
- 7 Clift RA, Buckner CD, Thomas ED *et al*. Marrow transplantation for chronic myeloid leukemia: a randomized study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. *Blood* 1994; **84**: 2036–2043.
 - 8 Mehta J, Powles RL, Mitchell P *et al*. Graft failure after bone marrow transplantation from unrelated donors using busulfan and cyclophosphamide for conditioning. *Bone Marrow Transplant* 1994; **13**: 583–587.
 - 9 Klein JL, Avalos BR, Belt P *et al*. Bone marrow engraftment following unrelated donor transplantation utilizing busulfan and cyclophosphamide preparatory chemotherapy. *Bone Marrow Transplant* 1996; **17**: 479–483.
 - 10 Topolsky D, Crilley P, Styler MJ *et al*. Unrelated donor bone marrow transplantation without T cell depletion using a chemotherapy only conditioning regimen. Low incidence of failed engraftment and severe acute GVHD. *Bone Marrow Transplant* 1996; **17**: 549–554.
 - 11 Bertz H, Potthoff K, Mertelsmann R, Finke J. Busulfan/cyclophosphamide in volunteers unrelated donor (VUD) BMT: excellent feasibility and low incidence of treatment-related toxicity. *Bone Marrow Transplant* 1997; **19**: 1169–1173.
 - 12 Przepiorka KM, Weisdorf D, Martin P. 1994 Consensus Conference on acute GVHD-grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
 - 13 Bearman SI, Appelbaum FR, Buckner CD *et al*. Regimen related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol* 1988; **6**: 1562–1568.
 - 14 McDonald GB, Hinds MS, Fisher LD *et al*. Venocclusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med* 1993; **118**: 255–267.
 - 15 Beatty PG, Ash R, Hows JM, McGlave PB. The use of unrelated bone marrow donors in the treatment of chronic myelogenous leukemia: experience of four centers. *Bone Marrow Transplant* 1989; **4**: 287–290.
 - 16 Santos GW, Tutschka Pj, Brookmeyer R *et al*. Marrow transplantation for acute non-lymphocytic leukemia after treatment with busulfan and cyclophosphamide. *New Engl J Med* 1983; **309**: 1347–1353.
 - 17 Tutschka PJ, Copelan EA, Klein, JA *et al*. Bone marrow transplantation for leukemia following new busulfan and cyclophosphamide regime. *Blood* 1987; **70**: 1382–1388.
 - 18 Sahebi F, Copelan E, Crilley P *et al*. Unrelated allogeneic bone marrow transplantation using high-dose busulfan and cyclophosphamide for preparative regimen. *Bone Marrow Transplant* 1996; **17**: 329–333.
 - 19 Ringden O, Ruutu T, Remberger M *et al* for the Nordic Bone Marrow Transplantation Group. A randomized trial comparing busulfan with total body irradiation as conditioning in allogeneic marrow transplant recipients with leukemia: a report from the Nordic Bone Marrow Transplantation Group. *Blood* 1994; **83**: 2723–2730.
 - 20 Andersson BS, Giral S, Tran H *et al*. I.V. busulfan, cytosin and allogeneic hematopoietic stem cell transplantation for advanced hematological malignancies. *Bone Marrow Transplant* 1999; **23** (Suppl. 1): 223.
 - 21 Gratwohl A, Hermans J, Niederwieser D *et al* for the Chronic Leukemia Working Party of the EBMT. Bone marrow transplantation for chronic myeloid leukemia: long term results. *Bone Marrow Transplant* 1993; **12**: 509–516.
 - 22 Devergie A, Apperley JF, Labopin M *et al* for the Chronic Leukemia Working Party of the European Group of Blood and Marrow Transplantation. European results of matched unrelated bone marrow transplantation for chronic myeloid leukemia. Impact of HLA class II matching. *Bone Marrow Transplant* 1997; **20**: 11–19.
 - 23 Sierra J, Storer B, Hansen J *et al*. Transplantation of marrow cells from unrelated donors for treatment of high risk acute leukemia: the effect of leukemia burden, donor HLA-matching and marrow cell dose. *Blood* 1997; **89**: 4226–4235.
 - 24 Kröger N, Zabelina T, Krüger W *et al*. Anti-human T-lymphocyte globulin (ATG) as part of the conditioning regimen in unrelated bone marrow transplantation of patients with CML in chronic or accelerated phase. *Blood* 1998; **92** (Suppl. 1): 578.
 - 25 Zander AR, Zabelina T, Löliger C *et al*. Bone marrow transplantation from mismatched unrelated donors. *Bone Marrow Transplant* 2000; **25** (Suppl. 1): 36.
 - 26 Zander AR, Zabelina T, Kröger N *et al*. Use of a five-agent GVHD prevention regimen in recipients of unrelated donor marrow. *Bone Marrow Transplant* 1999; **23**: 889–893.