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

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Between October 1988 and December 1992, 167 patients with leukemia receiving marrow transplants from HLA-identical donors and conditioned with cyclophosphamide (120 mg/kg) were randomized to additional treatment with either busulfan (16 mg/kg, n = 88) or TBI (n = 79). The busulfan-treated patients had an increased cumulative incidence of veno-occlusive disease of the liver, ie, 12% compared with 1% in the TBI group (P = .008). Furthermore, hemorrhagic cystitis occurred in 24% of the busulfan patients versus 8% in the TBI patients (P = .003). In patients with advanced disease beyond first remission or first chronic phase, transplantation-related mortality was 62% among the busulfan-treated patients compared with 12% among the TBI recipients (P = .002). These differences between the two groups were statistically significant in multivariate analysis. Seizures were seen in 6% of the busulfan-treated patients and were absent in the TBI group (P = .03). Grade II-IV of acute graft-versus-host disease (GVHD) was similar in the two groups, but grade III-IV and chronic disease was more common in the busulfan-treated group (P = .04). Death associated with GVHD occurred in 17% of the busulfan-treated group and 2% of the TBI group (P = .003).

Bone marrow transplantation (BMT) is well established for the treatment of hematologic malignancies. Survival after BMT is expected to be superior compared with conventional chemotherapy, although this has only been proven in a few prospective trials. The most commonly used ablative therapy in the preparation of patients for allogeneic BMT has been cyclophosphamide combined with total body irradiation (TBI). Apart from acute toxicity, TBI may induce late toxic effects, including pneumonitis, cataracts, endocrinologic disturbances, secondary malignancies, and, in children, decreased growth. Despite the heavy ablative therapy, relapse is one of the major causes of death in patients undergoing BMT. Increased chemotherapy dosage and/or TBI have resulted in a decreased risk of relapse, but at the price of more toxicity, resulting in an unchanged disease-free survival.

In patients in first remission of acute myeloid leukemia (AML), busulfan (16 mg/kg) combined with cyclophosphamide (200 mg/kg) resulted in patient survival similar to that seen with cyclophosphamide at 120 mg/kg combined with TBI. However, in the later stages of AML, toxic side-effects were common and survival was poorer. A combination of busulfan (16 mg/kg) with a lower dose of cyclophosphamide (120 mg/kg) has resulted in promising results in different types of leukemias and disease states with acceptable levels of toxicity. To challenge these promising data, the Nordic Bone Marrow Transplantation Group performed a prospective study comparing conditioning for hematologic malignancies using the same cyclophosphamide dosage (120 mg/kg) but randomizing patients to either busulfan (16 mg/kg) or TBI. We report the results in 167 consecutive patients with leukemia or lymphoma receiving marrow from HLA-identical related donors.

Patients treated with busulfan had a 3-year actuarial survival of 62%, which was worse than the 76% among those treated with TBI (P < .03). In multivariate analysis, poor survival was associated with advanced disease (P < .001), no posttransplant septicemia (P = .0006), grade II-IV GVHD (P = .006), and busulfan treatment (P < .02). The incidence of relapse did not differ between the two groups. Relapse-free survival was also similar in the two treatment groups on analysis of data from all patients, children, patients with early disease, and those with acute myeloid leukemia, acute lymphoblastic leukemia, and chronic myeloid leukemia. However, in adults (P = .05) and patients with advanced disease (P = .005), leukemia-free survival was significantly better in those treated with TBI. We conclude that patients treated with busulfan have more early toxicity and an increased transplant-related mortality in patients with advanced disease. TBI is therefore the treatment of choice, especially in adults and patients with advanced disease. However, busulfan is an acceptable alternative for patients with early disease and for those in whom TBI is not feasible.
Gothenburg. Consent was obtained from all patients or legal guardians. Each center reported the following number of patients: Helsinki (68), Huddinge (54), Copenhagen (20), Turku (12), Lund (11), and Gothenburg (2). Between October 1988 and December 1992, 167 patients were included in the trial; 88 were randomized to treatment with busulfan and 79 to treatment with TBI. The number of patients with early disease was 59 and 61 in the two groups, respectively. Patient characteristics in the two groups are given in Table 1.

**Conditioning regimen.** Busulfan (1 mg/kg) was administered four times per day for 4 consecutive days from day −8 to day −5. To prevent seizures, the patients received diazepam, clonazepam, or clorazepam during busulfan treatment. Cyclophosphamide (60 mg/kg) was administered on days −4 and −3. To prevent seizures, the patients received diazepam, clonazepam, or clorazepam during busulfan treatment. Cyclophosphamide (60 mg/kg) was administered on days −4 and −3. To prevent hemorrhagic cystitis, alkalinization and forced diureses were induced by giving 3 L fluid/m² intravenously (IV) for 24 hours during cytostatic drug treatment. During cyclophosphamide treatment, Uromitexan was administered (30% of the cyclophosphamide dose twice per day). All patients received 12 mg of methotrexate IT on two occasions before BMT. The TBI patients received cyclophosphamide (60 mg/kg) on 2 consecutive days and TBI according to the regimen at each specific center. All centers used linear accelerators. At Huddinge Hospital, 10 Gy TBI was administered in one treatment (4.0 cGy/min) on day −1 with the lungs shielded to receive no more than 9 Gy. At the other centers, a total of 11.3 to 12 Gy TBI was administered in 3 to 7 fractions (range, 4.0 to 12.7 cGy/min), and the lungs were shielded to receive between 9.0 to 10 Gy.

**Posttransplant immunosuppression.** The patients received 4 IV doses of methotrexate (MTX) combined with cyclosporine (CSA). CSA was discontinued from 2 to 12 months after BMT in the absence of graft-versus-host disease (GVHD). Patients with acute lymphoblastic leukemia (ALL) and patients with AML type M4 and M5 received IT MTX (12 mg) on day 32 and then every alternate week until day 102. Patients with a history of central nervous system (CNS) leukemia received in addition IT MTX every 8 weeks for 1.5 years. At Huddinge Hospital, MTX was administered IV once every week, starting when the number of polymorphonuclear cells (PMN) was more than 0.5 × 10⁹/L, at a dose of 10 mg/m² until 3 months after BMT. IV MTX was not administered during weeks when IT MTX was administered.

**Definitions and grading of complications.** Acute GVHD was graded from 0 to IV according to previous published criteria. Symptomatic hemorrhagic cystitis was defined as macroscopic hematuria. The definition of transfusion-dependent cystitis was an increase in bilirubin level greater than 2 mg/dL with at least two of three of the following: hepatomegaly, ascites, and greater than 5% body weight gain. Many patients also experienced cholecystitis and, in addition, VOD was also verified by biopsy and/or ultrasound. If an increase in bilirubin level and only one additional criterion was met, the additional increase in liver enzymes would indicate “suspected VOD” if GVHD and hepatitis could be excluded.

**Statistical analysis.** Analysis was performed January 31, 1993, with an observation time from 1 month to 50 months. Means were compared with Student’s t-test. Distribution was compared with the χ² test and corrected with the Yate’s method. Time to hemorrhagic cystitis, VOD, GVHD, death, relapse, and survival were analyzed by the life-table method. Differences in survival, etc., between the subgroups were studied using the log-rank test, taking censored data into account. Patients with incomplete follow-up were considered to be censored observations. Cox’s regression model was used for multivariate survival analysis. The covariates were examined without the influence of GVHD. With the addition of time-dependent variables such as engraftment, septicemia during the first month, and acute GVHD, survival was analyzed after more than 30 days and more than 90 days.

**RESULTS**

**Engraftment and transfusion.** Engraftment of PMN and reticulocytes were the same in both busulfan- and TBI-treated patients (Table 2). The need for erythrocyte and platelet transfusions was also similar in the two groups.

**Complications.** The incidence of seizures was 6% in the busulfan group compared with 0% in the TBI group (P =
VOD. VOD occurred in 12% of patients treated with busulfan compared with 1% of those who received TBI (P = .009, Table 2 and Fig 2). Proven VOD was 8% and 1% in the two groups, respectively (P = .017). Suspected VOD on weaker clinical grounds was seen in 3% of the busulfan group and in 0% of the TBI patients (P = .6). The following factors were of no importance in the occurrence of VOD: age, sex, diagnosis, disease status, splenectomy, transplant center, CMV serology, and GVHD. Treatment with busulfan versus that with TBI was the only prognostic factor associated with VOD. There was an association between hemorrhagic cystitis and VOD (P = .003).

Hemorrhagic cystitis. The overall incidence of symptomatic hemorrhagic cystitis was 24% in the busulfan patients compared with 8% in the TBI group (P = .003, Table 2 and Fig 3). The incidence of transfusion-dependent hemorrhagic cystitis was 3% and 1%, respectively (P = .3). In bivariate analysis, the following factors were not associated with hemorrhagic cystitis: age, sex, diagnosis, transplant center, CMV serology, splenectomy, and acute GVHD. Among patients with early disease, the incidence of hemorrhagic cystitis was 14%, compared with 29% in patients with advanced disease (P = .058). In multivariate analysis, hemorrhagic cystitis was associated with conditioning with busulfan (P = .0013) and advanced disease status (P = .002, Table 3).

Causes of death. There were 31 deaths in the busulfan group and 16 deaths among the recipients of TBI. The primary causes of death were the following in the busulfan/TBI groups: relapse (7/9), interstitial pneumonitis (6/3), infection (5/1), hemorrhages (2/2), acute GVHD (6/0), chronic GVHD (2/1), multiorgan failure (1/0), and other (2/0). Furthermore, acute GVHD contributed to the deaths of another 5 patients treated with busulfan but to no patient who received TBI. Chronic GVHD contributed to the death of 1 patient treated with busulfan. Death associated with GVHD was more common in the busulfan-treated group (17% vs 2% in the TBI group; P = .003). Multiorgan failure contributed to the deaths of 3 patients treated with busulfan and 1 patient treated with TBI. VOD contributed to death in 2 patients treated with busulfan.
Transplantation-related mortality. Death by causes other than relapse occurred in 28% of the busulfan recipients compared with 9% of the TBI patients \( (P = .006) \). This difference was caused by the fact that patients with advanced disease had an increased incidence of transplantation-related mortality using busulfan \( (62\% \text{ vs } 12\% \text{ with TBI}; \ P < .002, \text{ Fig } 4) \). Furthermore, donor age above 30 years was associated with increased transplantation-related mortality \( (P = .01) \). Recipient age, acute leukemia versus CML, CMV serology, time to engraftment, and transplant center had no impact on transplantation-related mortality. In multivariate analysis, factors associated with transplantation-related mortality were advanced disease \( (P < .0001) \), older donor age \( (P = .0002) \), and busulfan treatment \( (P = .05) \) (Table 4).

Survival. Overall outcome with regard to patient survival, relapse, and leukemia-free survival is summarized in Table 5. Actuarial 3-year survival of all patients was 62% in the busulfan group compared with 76% in the TBI patients \( (P < 0.03, \text{ Fig } 5) \). In patients with early disease, actuarial 3-year survival was similar in the two groups, but in patients with advanced disease, actuarial 3-year survival was 21% in the busulfan group versus 66% among the TBI patients \( (P = .002) \). The following factors were of no importance for patient survival: age, diagnosis, transplant center, CMV serology, and marrow cell dose. The following variables were associated with improved patient survival in univariate analysis: early leukemia \( (P < .0001) \), treatment with TBI \( (P = .018) \), CML versus acute leukemia \( (P = .019) \), no septicemia during the first months after BMT, and grade 0-1 acute GVHD \( (P = .002) \). In multivariate analysis, improved patient survival was significantly associated with early leukemia \( (P < .0001) \), absence of septicemia \( (P = .006) \), grade 0-1 GVHD \( (P = .006) \), and TBI \( (P = .02) \) (Table 6).

Relapse and relapse-free survival. The overall cumulative incidence of relapse did not differ regardless of diagnosis, disease status, or type of conditioning (Table 5 and Fig 6). Overall relapse-free survival for patients with early disease, AML, ALL, or CML did not differ between those treated with busulfan or those treated with TBI (Table 5). However, in patients with advanced disease, relapse-free survival was significantly better in those treated with TBI \( (P = .005, \text{ Fig } 7) \).

Outcome in adults and children. Patient survival at 3 years
was 62% in all 73 adults (>17 years) treated with busulfan, which was significantly worse than 79% of all adults treated with TBI (n = 68; \( P < .02 \)). The cumulative incidence of relapse was 23% and 24% in the two groups, respectively. Relapse-free survival was 54% and 68% in the two groups, respectively (\( P = .05 \)). In children (<18 years of age), patient survival and relapse-free survival at 3 years was 66% in the busulfan group (n = 15) compared with 67% in the TBI-treated children (n = 12; NS). The 3-year probability of relapse was 16% and 33% in the two groups, respectively (NS).

**DISCUSSION**

Several outcome variables were similar in patients conditioned with busulfan and TBI, eg, time to engraftment, transfusion requirements, septicemia, and interstitial pneumonitis. TBI dose and dose rate can influence the risk of interstitial pneumonitis. However, all our patients received either lung shielding, fractionated TBI, or both, which decreases the risk of interstitial pneumonitis. This trial shows that, with these precautions, the risk of interstitial pneumonitis using TBI is not worse than that with busulfan.

There was more grade III-IV acute GVHD and also chronic GVHD in the busulfan group, as compared with the TBI recipients (Table 2). The reason for this may be that busulfan causes more tissue toxicity, which, in turn, may lead to more severe acute GVHD and, subsequently, also chronic GVHD. Longer follow-up may further elucidate the risk of chronic GVHD using busulfan.

Patients treated with busulfan had an increased incidence of early toxicity. Thus, there was a significantly increased cumulative incidence of VOD, ie, 12% among the busulfan treated patients compared with 1% in the TBI patients (Fig 2). It is known that busulfan conditioning for BMT may cause VOD, particularly high busulfan levels in adults. Therefore, it is possible that individual dosing may prevent or decrease the risk of VOD on conditioning with busulfan.

Another major complication that was more common in the busulfan group was hemorrhagic cystitis (Fig 3). Hemorrhagic cystitis occurred in as many as 24% of the busulfan patients and 3% of these were transfusion dependent. Such an association between busulfan and hemorrhagic cystitis has not been reported previously. The reason for this may be that all our patients were treated with MTX in addition to CSA to prevent GVHD. Tutschka et al combined CSA with prednisolone and rarely saw hemorrhagic cystitis. It is possible that the combination of busulfan and MTX may be particularly harmful to the epithelium of the urinary tract. Furthermore, the late occurrence of hemorrhagic cystitis after 6 months, as seen in some of our patients, may have been overlooked in some studies.

Despite prophylaxis with anticonvulsive drugs, 6% of the busulfan-treated patients experienced seizures during therapy. This problem was not seen in any of the patients in the TBI group.

TBI is associated with several long-term side-effects, such as cataracts, secondary malignancies, endocrinologic disturbances, and, in children, decreased growth and dental development. With a longer follow-up we will perform a further analysis with special emphasis on these late effects. Alopecia is a problem seen in many of our patients treated...
with busulfan, in contrast to those treated with TBI. Longer follow-up is needed before this problem can be fully evaluated. Death by GVHD was more common in the busulfan group than among the TBI patients. This difference is caused by more frequent severe acute GVHD and chronic GVHD and may be associated with the increased toxicity induced by busulfan in various tissues resulting in, for instance, VOD and hemorrhagic cystitis. Taken together, these effects resulted in a significantly increased incidence of transplantation-related mortality among the busulfan-treated patients with advanced disease (P = .002, Table 4 and Fig 4).

Our study indicates that busulfan may be particularly unsuitable for patients with advanced disease who have received several courses of cytostatic drugs before BMT. A poor outcome for high-risk patients treated with busulfan was also reported by Santos et al.\textsuperscript{11} in that study, an increased dose of cyclophosphamide was administered (200 mg/kg, compared with 120 mg/kg in our study).

TBI was of importance for improved overall survival in multivariate analysis compared with busulfan (P = .02, Table 6). More important for survival was the disease status. Patients with early disease did significantly better than patients with more advanced disease, a fact known from previous studies.\textsuperscript{15,16} As expected, sepsis and GVHD were associated with increased mortality.\textsuperscript{14} In the comparison of patients with advanced disease, there were only 29 patients treated with busulfan and 18 with TBI. Furthermore, a 61% 3-year probability of leukemia-free survival is better than expected for patients with advanced leukemia, and it cannot be excluded that this may be an artefact of the low sample size. Therefore, these survival data must be interpreted with caution.

When different types of leukemias were analyzed separately, there were no differences between the TBI and busulfan groups (Table 5). This finding is in contrast to a randomized French multicenter study in which AML patients in first remission treated with TBI had an actuarial 3-year probability of survival of 72% that was significantly better than the probability of survival of 47% among patients treated with busulfan (P < .01).\textsuperscript{22} This discrepancy between the two studies may not be significant. The same busulfan protocol was used in both studies, yet the disease-free survival in the French report was 47%, compared

### Table 5. Actuarial Patient Survival, Relapse, and Leukemia-Free Survival in All Patients Randomized to Treatment With Busulfan or TBI

<table>
<thead>
<tr>
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<th>Busulfan (n = 88)</th>
<th>TBI (n = 79)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Patient survival, 3 yrs</td>
<td></td>
<td></td>
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<tr>
<td>All patients</td>
<td>62%</td>
<td>76%</td>
<td>.026</td>
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<tr>
<td>Early disease*</td>
<td>83% (n = 59)</td>
<td>80% (n = 61)</td>
<td>.8</td>
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<tr>
<td>Advanced disease†</td>
<td>21% (n = 29)</td>
<td>66% (n = 18)</td>
<td>.002</td>
</tr>
<tr>
<td>Relapse, 3 yrs</td>
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<td></td>
<td></td>
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<tr>
<td>All patients</td>
<td>22%</td>
<td>26%</td>
<td>.9</td>
</tr>
<tr>
<td>Early disease</td>
<td>14%</td>
<td>25%</td>
<td>.29</td>
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<tr>
<td>Advanced disease</td>
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<td>31%</td>
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<td>Relapse-free survival, 3 yrs</td>
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<tr>
<td>All patients</td>
<td>56%</td>
<td>67%</td>
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<tr>
<td>Early disease</td>
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<td>Advanced disease</td>
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<tr>
<td>AML</td>
<td>61% (n = 37)</td>
<td>64% (n = 32)</td>
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<tr>
<td>ALL</td>
<td>36% (n = 17)</td>
<td>50% (n = 20)</td>
<td>.29</td>
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<tr>
<td>CML</td>
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<td>83% (n = 26)</td>
<td>.25</td>
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<tr>
<td>AML first remission</td>
<td>83% (n = 25)</td>
<td>60% (n = 26)</td>
<td>.22</td>
</tr>
<tr>
<td>CML first chronic phase</td>
<td>84% (n = 22)</td>
<td>81% (n = 24)</td>
<td>.56</td>
</tr>
<tr>
<td>ALL first remission</td>
<td>39% (n = 11)</td>
<td>41% (n = 11)</td>
<td>.89</td>
</tr>
</tbody>
</table>

* Acute leukemia and lymphoma in first remission and CML in first chronic phase.
† Advanced disease; later stages.
with 83% in our first complete remission (CR) patients (Table 5). Furthermore, the 73% disease-free survival in the French patients with AML in first remission treated with TBI is probably not significantly different from the 58% observed in the Nordic study. This shows the importance of repeated prospective large-scale randomized trials. Additional randomized trials or large multicenter analyses may be needed to determine whether TBI is superior to busulfan as conditioning in patients with AML in first CR. Furthermore, the type of TBI has also to be considered.25,30

Recipient age had no impact on survival in this study, in contrast to many previous reports in which children appear to have a better prognosis after BMT than adults.23-25 One reason for this discrepancy may be that only a few children (n = 27) were included in this trial and therefore a survival difference could not be detected. Another explanation may be that recently improved prevention of GVHD using MTX combined with CSA, instead of previous monotherapy with either drug alone, has increased survival in adults, but not in children.26,27

To conclude, from this study of 167 patients, busulfan treatment was associated with several unwanted toxic side-effects, eg, severe acute GVHD, chronic GVHD, VOD, and hemorrhagic cystitis. This resulted in an increased transplantation-related mortality in the busulfan group (P = .05, Table 4) that was striking in patients with advanced disease (P = .002). Relapse-free survival was decreased in busulfan-treated patients with advanced disease (P = .005) and in adults (P = .05). Therefore, we recommend TBI as conditioning in these groups. Furthermore, poor survival was associated with busulfan treatment in multivariate analysis (P < .02). In the absence of TBI, or in unsuitable patients who have been previously treated with high doses of irradiation for instance towards the CNS, busulfan is an acceptable alternative, especially in early leukemia, with survival rates similar to those obtained with TBI.

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REFERENCES

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