Acute Toxicity and First Clinical Results of Intensive Postinduction Therapy Using a Modified Busulfan and Cyclophosphamide Regimen With Autologous Bone Marrow Rescue in First Remission of Acute Myeloid Leukemia

By Dietrich W. Beelen, Klaus Quabeck, Ullrich Graeven, Herbert G. Sayer, Hossam K. Mahmoud, and Ulrich W. Schaefer

The combination of high-dose busulfan (16 mg/kg) and 200 mg/kg cyclophosphamide is gaining increasing significance as a preparative regimen prior to autologous, syngeneic, or alllogeneic marrow transplantation. A new regimen of high-dose busulfan in conjunction with a reduced dose of 120 mg/kg cyclophosphamide has recently been described as a preparative regimen prior to allogeneic transplantation. To determine the drug-related nonhematologic toxic effects of this new regimen without confounding factors associated with allogeneic transplantation, we conducted a pilot study using this new regimen in 20 patients with acute myeloid leukemia (AML) in first remission prior to autologous unpurged marrow transplantation. All patients experienced transient non-life-threatening acute drug-related toxicity with skin reactions in 20 (100%), nausea and vomiting in 20 (100%), oral mucositis in 18 (90%), hepatic functional impairment in 17 (85%), hemorrhagic cystitis in three (15%), and generalized seizures in two (10%) of these patients, respectively. Two procedural, fatal complications resulted from infectious causes that were not directly related to the speed of hematopoietic reconstitution or the toxicity of the preparative regimen. The 3-year event-free survival estimate (55% ± 11%) and probability of leukemic recurrence (38% ± 11%) attained with this new regimen in recipients of autografts in first remission of AML are promising and challenge comparisons with preparative regimens employing combinations of cytotoxic agents or total body irradiation (TBI).

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special concern in a therapeutic approach, the significance of which has still to be established.\(^4\) Second, in contrast to allogeneic BMT, intensive immunosuppression, which per-
ferentially is achieved by CY, is not required prior to autologous BMT. In addition, it is presumed that the antileu-
kemic effect of this combination depends predominantly on the myelotoxic properties of busulfan. We report our experi-
ence using this new regimen in 20 consecutive patients, with special emphasis on acute and subacute toxic effects in the setting of autologous BMT.

**MATERIALS AND METHODS**

**Patients and characteristics.** Patients with AML in first remission who were referred to our institution for autologous BMT were included after written informed consent had been obtained for all aspects of the procedure following guidelines approved by the Committee on Treatment of Human Subjects in Research at the University Hospital Essen. The 20 patients presented in this analysis received autologous BMT between February 1986 and June 1988. Persistent organ functional impairment as well as a considerably reduced capacity of the patients’ marrow cells to form granulocyto-
monocyte colonies (CFU-GM) in semisolid culture assays (defined as less than 10% of colony-forming units in relation to our laboratory standard) were regarded as medical exclusion criteria. During the time interval covered by this analysis, only one patient who presented with elevated liver enzymes was excluded from autologous BMT. In this patient leukemic infiltrates were later demonstrated by liver biopsy. No exclusion was necessary due to a reduced proliferative capacity of the patients’ marrow. Any patient selection with regard to the time interval to achieve remission or the duration of remission prior to autologous BMT was avoided, and the latter time interval was predominantly influenced by reasons out of control of the transplant center. None of the patients had an HLA-identical sibling donor who would have qualified for allogeneic BMT. Prior to marrow harvest, the remission status was confirmed by bone marrow (BM) aspirate and BM biopsy. With rare exceptions, the upper age limit for autologous BMT at our department is 50 years.

The median age of the 20 patients was 40 (range 16 and 53) years. Eight patients were female, and 12 patients were male. Seventeen patients had received cytosine arabinoside (ARA-C), daunorubicin (DNR), and thioguanine (TG) according to the TAD-9 protocol (100 mg/m\(^2\)/d ARA-C as continuous infusion, day 1 and day 2, 30-minute infusions every 12 hours, days 3 through 8; 60 mg/m\(^2\)/d DNRC intravenously [IV] days 3, 4, and 5; 100 mg/m\(^2\)/d TG orally every 12 hours, days 3 through 9) as induction chemother-
therapy.\(^4\) Definition of remission met the criteria of the Cancer and Leukemia Study Group B.\(^9\) Complete remission was achieved after one course in six (UPN 145, UPN 167, UPN 176, UPN 200, UPN 249, UPN 265) and two courses in six (UPN 141, UPN 189, UPN 193, UPN 223, UPN 239, UPN 251) of these patients. One patient (UPN 256) attained complete remission after one course of the TAD-9 regimen followed by a combination of high-dose ARA-C (3 g/m\(^2\)/d IV every 12 hours for three days) and mitoxantrone (10 mg/m\(^2\)/d IV, days 3 through 5) according to the HAM protocol.\(^1\) All patients who had entered remission after the first course of chemotherapy received a second induction course according to the TAD-9 regimen (UPN 145, UPN 176, UPN 249, UPN 265) or the HAM regimen (UPN 167, UPN 200). In four patients only a partial remission with a residual marrow infiltration over 10% of myeloblasts could be attained after two (UPN 212, UPN 234) or three consecutive courses (UPN 140, UPN 185) of the TAD-9 regimen. These patients entered complete remission after one additional course of the TAD-9 regimen (UPN 185, UPN 212); four courses of a combination of CY, vincristine, ARA-C and prednisone (COAP; UPN 40); or one course of HAM followed by a combination of ARA-C, etoposide, and amraspam (AYA; UPN 234), respectively. In three patients remission induction chemotherapy consisted of a combination of high-dose ARA-C with epirubicin (HAE; UPN 157) or ARA-C and DNR (AD; UPN 221, UPN 247). The median duration from diagnosis to complete remission was 37 (range 16 and 157) days. Postinduction chemotherapy consisted of one to six courses of maintenance therapy according to a study protocol for the treatment of adult AML in the FRG in 11 patients.\(^5\) These mainte-
nance courses were based on ARA-C (100 mg/m\(^2\)/d subcutaneously [SC] every 12 hours, days 1 through 5) in combination with TG (200 mg/m\(^2\)/d orally, days 1 through 5), DNR (45 mg/m\(^2\)/d IV, days 3 and 4) or CY (1,000 mg/m\(^2\)/d IV, day 3), delivered sequentially every 4 weeks. Consolidation chemotherapy was performed according to the TAD-9 regimen in six patients. Three patients received HAE, HAM, or AYA consolidation therapy, respectively. Patient charac-
teristics and clinical data of the disease course before autologous BMT are summarized in Table 1.

**Harvest and processing of bone marrow cells.** Marrow harvest was performed after a median interval of 173 (range 76 and 410) days from entering complete remission. A median of 1.8 (range 1.2 and 2.6) x 10\(^6\) nucleated BM cells in a total volume between 892 and 2,317 (median 1,749) mL were removed under general anes-
thesia by multiple aspirations from the posterior iliac crests and collected in Hank’s buffered salt solution (HBSS; Seromed, Bio-
chrom KG, Berlin, FRG) containing heparin (Liquemin, Hoffmann-
La Roche AG, Grenzach-Wyhlen, FRG). In the first 12 patients the nucleated cells were separated from the blood-marrow mixture by centrifugation at 1,000 g for 20 minutes in 50-mL tubes (Falcon Laboratories, Becton-Dickinson, Heidelberg, FRG). After remixing of the residual rod cell pellet and the plasma, a second buffy coat was extracted. In the following patients mononuclear cells were sepa-
rated by continuous-flow centrifugation on an IBM 2997 blood cell separator as previously described.\(^13\) A median of 1.42 (range 0.44 and 3.66) x 10\(^8\) cells/kg body weight were recovered after separation. The concentrated cells were resuspended in HBSS containing 5,000 IU heparin, 20% AB plasma or autologous plasma, and 10% dimethylsulfoxide (Merck, Darmstadt, FRG) to yield a final volume of 260 mL. The final cell concentration constantly exceeded 5 x 10\(^8\) cells/mL. One hundred thirty-milliliter aliquots were transferred to polyeolin freezing bags (Delmed Inc, Canton, MA). The cells were frozen at a rate of 1°C/min to –40°C and then at 3°C/min to –80°C using a controlled-rate freezer (Kryo 10 Series, chamber Model 10-16, controller model 20-21, Planer products LTD, Middle-
sex, Great Britain). The bags were stored in the liquid phase of a liquid nitrogen freezer (Model CS 320 LR and LPL 200, Union Carbide Corp, Indianapolis).

**Hematopoietic progenitor cell assay.** For evaluation of frequen-
cies of CFU-GM, 2 x 10\(^8\) marrow mononuclear cells separated by ficoll-
hyapou dense gradient centrifugation were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) with 10% fetal calf serum (FCS) and 0.25% agar, using human blood mononuclear cells as a source of colony-stimulating activity. To estimate the recovery of CFU-GM after freezing and thawing, the thawed cells were cultured directly without further concentration. Colonies of greater than 50 cells were scored after 14 days incubation at 37°C, 10% CO\(_2\), and high humidity (Table 2).

**Preparative regimen and autologous BMT.** The median time interval between marrow harvest and beginning of the preparative regimen was six (range 2-44) days. No patient received further chemotherapy during this interval. The myeloablative regimen was carried out as described for allogeneic BMT by Tutschka et al\(^4\) with the exception that the doses of busulfan and CY were not corrected for the patients’ ideal body weight. Busulfan was given from day –7
to day – 4 in four daily doses of 1 mg/kg for a total dose of 16 mg/kg body weight. Oral phenytoin (100 mg tid) was added on the days of busulfan administration to prevent convulsions. On day – 3 and day – 2 CY at a daily dose of 60 mg/kg body weight was administered as a one-hour infusion. For prophylaxis of hemorrhagic cystitis, all patients received on the days of CY administration 60 mg/kg body weight sodium 2-mercaptoethanesulphonate (mesna) in five divided doses in combination with forced diuresis and IV HCO₃ supplementation if the urine pH declined below 7. On day 0, the frozen marrow was thawed by a stepwise dilution technique for removal of dimethylsulfoxide as described by Schaefer. To achieve decontamination of the gastrointestinal (GI) tract. Parenteral nutrition solutions, systemic antibiotics, and blood products were administered through a central venous line over a one-hour period. Prophylactic oral nonabsorbable antibacter-ia were given until a self-sustaining platelet count over 20 × 10⁹/L was reached, and RBC concentrates were administered when the hemoglobin concentration declined below 8 g/L. Antibiotics and isolation procedures were maintained until the absolute neutrophil counts exceeded 1.0 × 10⁹/L and the patients were afebrile and without signs of infection.

**Supportive therapy.** All patients were hospitalized in single reverse-isolation rooms. Prophylactic oral nonabsorbable antibiotic and antifungal agents were administered as well as autoclaved food were given to achieve decontamination of the gastrointestinal (GI) tract. Parenteral nutrition solutions, systemic antibiotics, and blood products were administered through a central venous line. Fever over 38.5°C during the phase of marrow aplasia was treated with broad-spectrum antibiotics. If fever persisted despite an absolute neutrophil count over 1.0 × 10⁹/L, systemic amphotericin-B therapy was initiated empirically. For blood-product substitution, preparations from cytomegalovirus (CMV) antibody-negative volunteer donors were used exclusively. All blood products were irradiated with 1.5 to 2.0 Gy to prevent transfusion-induced graft-vs-host disease (GVHD). Platelet transfusions were given until a self-sustaining platelet count over 20 × 10⁹/L was reached, and RBC concentrates were administered when the hemoglobin concentration declined below 8 g/L. Antibiotics and isolation procedures were maintained until the absolute neutrophil counts exceeded 1.0 × 10⁹/L and the patients were afebrile and without signs of infection.

**Statistical analysis.** The event-free survival estimate and the probability of leukemic recurrence were calculated by the product-limit estimates of Kaplan and Meier. For estimation of the probability of leukemic recurrence, patients were treated as censored observations if they died in continuous remission. The influence of delayed BMT on the time interval until relapse occurred was analyzed by correlating this time interval with the remission duration prior to BMT or the time to achieve complete remission. To analyze the speed of hematopoietic reconstitution, the individual time intervals for attaining a sustained peripheral blood cell count over a given endpoint were used to calculate Kaplan and Meier product-limit estimates of reaching this endpoint on a given day in the post-transplant period. Endpoints in this analysis were a white blood cell count over 100 × 10⁹/L and a platelet count in excess of 20 × 10⁹/L and 50 × 10⁹/L, respectively. Besides correlation analysis, a time-dependent, stepwise, proportion-hazards general-linear model was used to look for associations between the described endpoints of hematopoietic reconstitution and different parameters. These parameters included the time interval to attain complete remission, the duration of remission before
Table 2. Hematologic Reconstitution and Clinical Results After Autologous Transplantation

<table>
<thead>
<tr>
<th>UPN</th>
<th>CFU-GM Infused (x 10^6/kg)</th>
<th>Time to Attain ANC &gt;0.5 &gt;1.0 &gt;20 &gt;50 Platelets Days Days Days Days</th>
<th>Time to Relapse After ABMT Days</th>
<th>Remission Duration After ABMT Days</th>
<th>Survival After ABMT Days</th>
<th>Current Status (Cause of Death)</th>
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<td>140</td>
<td>6.3</td>
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<td>1108</td>
<td>1265</td>
<td>1108</td>
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<td>141</td>
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<td>61</td>
<td>404</td>
<td>1089</td>
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<tr>
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<td>-</td>
<td>1055</td>
<td>1419</td>
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<tr>
<td>167</td>
<td>1.0</td>
<td>35 63 79 93</td>
<td>-</td>
<td>894</td>
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<td>894</td>
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</tr>
<tr>
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<td>33 51 19 31</td>
<td>-</td>
<td>752</td>
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<tr>
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<td>193</td>
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<td>235</td>
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</tr>
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<tr>
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<td>265</td>
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<td>38 53 - -</td>
<td>99</td>
<td>99</td>
<td>226</td>
<td>260</td>
</tr>
</tbody>
</table>

Abbreviations: ANC, absolute neutrophil count; ABMT, autologous BMT; a/w, alive and well.

RESULTS

Posttransplant clinical course and hematologic toxicity. Nineteen of the 20 patients (95%) survived long enough to be evaluable for engraftment and hematopoietic reconstitution. One patient (UPN 193) died from an overwhelming sepsisemia with Streptococcus sanguis eight days after autologous BMT during marrow aplasia. Hematologic toxicity was severe and life threatening in all patients and occurred with an average onset at day 7 after completion of the preparative regimen. All patients experienced febrile episodes with body temperature rising beyond 38.5°C for a median time of eight (range 2 to 32) days. In eight of 20 patients (40%), a total of 15 blood cultures were positive for bacteria or fungi (coagulase-negative staphylococci n = 10, Corynebacteria n = 2, Streptococcus sanguis n = 1, Pseudomonas maltophilia n = 3, Candida albicans n = 1), and in three of these cultures growth of several organisms could be demonstrated. The median duration to reach peripheral blood levels of neutrophils over 0.5 x 10^9/L was 30 (range 20 to 53) days. A neutrophil count in excess of 1.0 x 10^9/L was attained at a median interval of 38 (range 26 to 77) days after autologous BMT. Recovery of platelets was generally slow. Persistent platelet counts under 20 x 10^9/L were associated with early recurrence of leukemia in one (UPN 265) and fatal bronchopneumonia in another patient (UPN 249). These two patients were excluded from the analysis of platelet recovery. In the remaining 17 patients, self-sustaining platelet counts over 20 x 10^9/L were reached between 11 and 128 (median 47) days. Despite lasting thrombocytopenic periods in most patients, only minor bleeding complications were observed. Platelet counts exceeding 50 x 10^9/L were achieved between 14 and 251 (median 63) days after BMT (Fig 1).

All patients required platelet and RBC transfusions for median intervals of 50 (range 14 and 101) and 48 (13 and 125) days, respectively. A median number of 20 (range 6 to 65) thrombapheresis preparations and 15 (range 6 to 31) packed red cell concentrates were needed for hemosubstitution after BMT.

We were unable to demonstrate any significant association between the time to attain each of these peripheral blood cell counts and the number of nucleated or colony-forming marrow cells infused, the time to achieve complete remission, or the duration of remission prior to harvest by multivariate analysis. With the exception of one patient (UPN 239) who never achieved normal peripheral platelet counts as a consequence of platelet-specific antibodies developing after autologous BMT, all patients had normal peripheral blood cell counts at time of analysis or until leukemic relapse occurred. Late infectious complications were restricted to one patient (UPN 234) who developed recurrent CMV-associated interstitial pneumonia.

Nonhematologic toxicity. Although all patients experienced manifestations of drug-related toxicity, the preparative regimen was relatively well tolerated. The majority of nonhematologic toxic effects occurred within the first 4 weeks after the start of the myeloablative therapy. Despite
A MODIFIED BUSULFAN/CY REGIMEN BEFORE ABMT

Fig 1. Cumulative probability of reaching the endpoints of peripheral blood cell recovery: granulocyte counts >0.5 × 10^9/L (--), granulocyte counts >1.0 × 10^9/L (——), platelet counts >20.0 × 10^9/L (-----), platelet counts >50.0 × 10^9/L (· · · · · · · · · · · · · ·).

an antiemetic prophylaxis, all 20 patients developed moderate-to-severe nausea and vomiting following the administration of CY. On the days of busulfan administration, only mild nausea in a minority of these patients was noted. Noninfectious oral mucositis developed in 18 patients (90%), which was scored as grade 1 in seven patients (35%) and as grade 2 to 3 in 11 patients (55%), respectively. Severe pain of the oral cavity or dysphagia precluding oral food intake made parenteral nutrition and fluid supplementation mandatory in all patients over time periods of 1 to 3 weeks. Diarrhea was not a prominent feature and was generally mild in symptomatic patients. Mild to moderate erythema and desquamation of intertriginous or mechanically burdened skin areas were noted in all patients. This was accompanied by a marked hyperpigmentation in two patients (UPN 141, UPN 251). These changes were transient and resolved spontaneously within 2 to 4 weeks after BMT.

Hepatic functional abnormalities were noted in a preponderant proportion of patients. Seventeen patients (85%) had transient elevations in SGOT/SGPT levels concomitant with the development of hyperbilirubinemia in 13 patients (65%). All of the abnormal levels improved to normal within a median time interval of ten (range 7 to 20) days for transaminases and of ten (range 1 to 51) days for bilirubin, respectively. None of these patients showed clinical signs of hepatic veno-occlusive disease.

Three patients (UPN 212, UPN 223, UPN 249) (15%) developed macroscopic hematuria in conjunction with dysuria and urinary frequency starting at 3, 4, and 7 weeks after the end of the preparative regimen. Since infectious or other causes for these symptoms could be excluded, these patients were classified as having toxic, hemorrhagic cystitis.

With regard to CNS toxicity, two patients (UPN 251, UPN 256) (15%) had a generalized seizure occurring on the days of busulfan administration despite prophylactic phenytoin treatment, and one patient (UPN 221) had a transient episode of acute obtundation after the first dose of CY. All three patients had no history of neurologic disturbances or convulsions, and metabolic causes of these events could be excluded. The recovery was complete in these three patients, and no further episodes have been noted.

Pulmonary symptoms of toxicity were not observed. One patient (UPN 234) developed nonfatal recurrent CMV interstitial pneumonia, and another patient (UPN 249) had to be treated by long-term mechanical ventilation due to acute respiratory failure caused by bilateral bronchopneumonia, from which he did not recover. In both patients slight lung fibrosis could be demonstrated histologically by transbronchial biopsy or at autopsy. Although previous busulfan treatment could not be excluded as a contributing factor for the development of lung fibrosis in these patients, it appears more likely that these changes were primarily initiated by
recurrent viral lung disease and the long-term respirator therapy, respectively. Renal, cardiac, or other complications that were unequivocally attributable to drug-related toxic effects were not observed in the post-transplant period (Table 3).

### Event-free survival and leukemic relapse
With a median observation period of 20 (range 11 to 36) months, 11 of 20 (55%) patients remain alive and well in unmaintained complete remission, resulting in a product-limit event-free survival estimate of 55% (SE + 11%) at 36 months after BMT. Two patients (10%) have died from nonleukemic causes, and in seven patients leukemic relapse occurred between 2 and 11 (median 8) months after BMT. The cumulative probability of leukemic recurrence is 38% (SE ± 11%; Fig 2). Four of the relapsing patients are still alive with a median follow-up of 11 (range 8 to 36) months. The overall survival estimate is 75% (SE ± 10%) at 36 months after BMT. There was no significant correlation between the time to achieve complete remission, the remission duration prior to BMT, and the time interval until relapse occurred.

### DISCUSSION
The combination of high-dose oral busulfan and IV CY gains increasing significance as a preparative regimen for marrow graft recipients. A number of clinical trials using this combination in patients with acute myeloid leukemia speaks well for an antileukemic efficacy, which appears comparable or even superior to that of regimens employing TBI in combination with CY prior to allogeneic, syngeneic, or autologous BMT. Without exception, these trials used a cumulative IV dose of 200 mg/kg body weight CY administered in four doses of 50 mg/kg over four days preceded by four oral doses of 1 mg/kg body weight busulfan daily on four consecutive days. It became apparent, however, that the high complication rate associated with this regimen in patients with AML receiving allografts from histocompatible sibling donors in first remission precluded an advantage with regard to long-term disease-free survival over TBI-containing regimens. CY was primarily introduced into this combination as a highly effective immunosuppressive agent that allows stable allogeneic engraftment. In the setting of autologous BMT as consolidation therapy for patients with AML in first remission, intensive and prolonged immunosuppression may be inadvisable by exposing the patient to a greater risk of infectious complications. Since it is broadly accepted that the myelotoxic properties of busulfan are the major determinant of the antileukemic efficacy of the busulfan/CY combination, the question has to be raised as to whether a dose reduction to the “standard dose” of 120 mg/kg CY, as generally used in combinations with TBI, would decrease the rate of therapy-associated complications without compromising the antileukemic efficacy of this regimen. This question is further substantiated in light of encouraging experiences recently reported with a new regimen using 16 mg/kg busulfan and 120 mg/kg CY in leukemia patients before allogeneic BMT. This report suggested a very low rate of therapy-related complications, and the 3-year actuarial relapse-free survival probability compared favorably with that reported for TBI-containing regimens.

This is the first report using this new regimen with autologous marrow support as consolidation therapy for patients with AML in first remission. For two principal reasons no attempt was made in our study to eliminate residual clonogenic leukemia cells from the marrow graft. First, despite high numbers of patients treated and numerous reports of clinical results that appear to be suggestive for efficacy of ex vivo marrow “purging” in first or second remission of AML, there is currently no proof for an increased antileukemic effect or improved survival after

### Table 3. Acute and Subacute Nonhematologic Toxicity of the Preparative Regimen

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Grade 0 (%)</th>
<th>Grade 1 (%)</th>
<th>Grade 2 (%)</th>
<th>Grade 3 (%)</th>
<th>Grade 4 (%)</th>
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<td>Liver</td>
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<td>2.6-5 x N</td>
<td>5.1-10 x N</td>
<td>&gt;10 x N</td>
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<tr>
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<td>11/20</td>
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<td>-</td>
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<tr>
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<td>10/20</td>
<td>4/20</td>
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<tr>
<td>Renal</td>
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<td>2.6-5 x N</td>
<td>5.1-10 x N</td>
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<td>20/20</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Creatinine</td>
<td>20/20</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Bladder</td>
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<td>Neurotoxicity</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>State of consciousness</td>
<td>17/20</td>
<td>1/20</td>
<td>-</td>
<td>-</td>
<td>2/20</td>
</tr>
</tbody>
</table>

*Upper limit of normal.
†Values represent proportion of patients.
A MODIFIED BUSULFAN/CY REGIMEN BEFORE ABMT

1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
TIME (MONTHS)

Fig 2. Kaplan-Meier product-limit estimates of event-free survival (-----) and leukemic recurrence (· · · · · · · · · · ) after a myeloablative regimen of busulfan 16 mg/kg and CY 120 mg/kg followed by autologous marrow transplantation.

autologous BMT using "purged" marrow.7,10 Second, it was our goal to evaluate the regimen-related toxic effects without confounding factors theoretically attributable to ex vivo treatment of the marrow graft that may increase the overall toxicity of the procedure.

Our results indicate that this preparative regimen has significant, albeit manageable, nonhematologic toxic effects. Nausea and vomiting, oral mucositis, toxic skin reactions, as well as mild diarrhea were frequent but uniformly transient and non-life threatening. With regard to these drug-related toxicities, our experiences compare favorably with the toxic effects of one trial in a heterogeneous group of patients using the higher dose of CY with busulfan prior to syngeneic or autologous BMT.5 With the exception of the profound vomiting seen with the acute administration of CY, similar GI and dermatologic side effects have previously been described with high-dose single-agent busulfan followed by autologous marrow support in the treatment of solid tumors in which the maximum tolerated dose of busulfan has been demonstrated to be 16 mg/kg, thus confirming the nonoverlapping toxicity of these agents.20

Hepatic functional abnormalities were expected and manifested as transient elevations in transaminases or bilirubin. No episodes of hepatic veno-occlusive disease or life-threatening liver toxicity occurred in our study, which is in contrast to the frequency of these complications reported in two different trials in which 16 mg/kg busulfan with the higher dose of CY had been used prior to syngeneic or autologous BMT.5,6 Taken together, at least five out of 28 evaluable patients (18%) described in these trials developed hepatic veno-occlusive disease or toxic liver failure. In three of these five patients, liver failure was the primary cause of death, accounting for a mortality rate of 10%. Although the significance of the apparent lack of severe liver failure associated with the new regimen is not clear, it might be speculated that the lower dose of CY attributed to a decreased cumulative liver toxicity. Hepatic veno-occlusive disease is not unique to high-dose busulfan and CY. It has also been noted with other myeloablative regimens and may reflect the cumulative effects of previous intensive cytotoxic therapy, undetected viral hepatitis, and individual differences in absorption or metabolism of the high doses of preparative agents as has been presumed by Lu.5 Nevertheless it is of note that only one out of 50 leukemia patients (2%) receiving the lower dose of CY in conjunction with 16 mg/kg busulfan prior to allogeneic BMT developed transient, nonfatal, hepatic veno-occlusive disease. Since the latter study included a high proportion of heavily pretreated and multitransfused patients, it appears unlikely that they had been at a substantially lower risk of developing this complication.8 It further
has to be taken into account that patients receiving allografts seem to have a higher probability of developing hepatic veno-occlusive disease. Therefore it appears justified to assume that the lower dose of CY has contributed to a reduced liver toxicity.

Hemorrhagic cystitis occurred in three patients despite mesna prophylaxis on the days of CY administration. Toxic effects on the urinary bladder are a long-recognized feature of the busulfan/CY combination. Two trials using this combination have reported frequencies of clinically significant hemorrhagic cystitis in the order of 10% to 20%. Hemorrhagic cystitis is a rare complication of conventional busulfan therapy, and the histologic changes of the bladder wall seen in these cases revealed generalized cystitis that was identical to CY and irradiation-induced cystitis. The dose-dependent toxic effects of acrolein and 4-hydroxy-cyclobiphosphamide, the major urinarily excreted metabolites of CY, on bladder epithelial cells are well established. Synergistic or cumulative toxicity of both agents or their metabolites on the bladder wall might therefore have promoted this complication despite mesna prophylaxis. An association between hemorrhagic cystitis and viruria with human polyomavirus has also been described in marrow graft recipients. Despite equivalent doses of CY, the incidence of hemorrhagic cystitis has been found to be higher after allogeneic as compared with autologous BMT, which has been explained by differences in active infection rates. In considering the late onset of hemorrhagic cystitis in our patients, it might be speculated that an as yet unidentified pathogen might at least in part have contributed to this complication.

CNS toxicity manifested as a single, generalized seizure in two patients and a transient episode of acute obtundation in a third patient. Generalized seizures with a combined busulfan/CY regimen have been reported previously, and most transplant centers are now using prophylactic anticonvulsants during administration of this regimen. Since we did not monitor phenytoin plasma levels on the days of seizures, we cannot exclude that insufficient levels allowed these events.

Pulmonary toxicity was not encountered in our study. Most importantly, we observed no case of idiopathic interstitial pneumonia, which is commonly thought to represent treatment toxicity. One patient (UPN 234) who had already suffered from this complication during induction chemotherapy developed recurrent, nonfatal CMV interstitial pneumonia after BMT. In a recent retrospective single-center analysis including 36 autografted patients with acute leukemia, the overall incidence of interstitial pneumonia was found to be 11% after a preparative regimen with high-dose busulfan and CY, and only one of these 36 patients (3%) was reported with idiopathic interstitial pneumonia. Whether busulfan, an agent capable of inducing acute, diffuse, interstitial pulmonary fibrosis, might add to an increased risk of toxic pulmonary complications when given as part of high-dose regimens is not known. In a phase I-II clinical trial of high-dose, single-agent busulfan with autologous marrow support, pulmonary function testing, including diffusing capacity for carbon monoxide and arterial blood gases before and after therapy, were not significantly different in four evaluable patients. Idiopathic interstitial pneumonia in the setting of autologous BMT has also been noted with similar or even higher frequencies after preparative regimens containing TBI in conjunction with CY. Thus, taking the available clinical experiences together, there is currently no clear-cut evidence that the combination of high-dose busulfan and CY leads to an increased risk of pulmonary toxicity after autologous BMT.

Although direct evidence for the marrow-lethal dose in humans is not available, the dose of busulfan used in the present study is generally considered to be marrow ablative. This assumption is derived from experimental data of a rat model in which this agent has been shown to produce fatal marrow aplasia with an LD_{100} dose of 30 mg/kg that can be reversed by BMT of syngeneic marrow. Taking commonly accepted conversion factors as a basis, this dose would be equivalent to a dose on the order of 6 mg/kg in humans, which is less than half of the dose used in our preparative regimen. Further evidence for the myeloablatative properties of the new regimen comes from the observation that all of 48 evaluable patients had sustained engraftment of all hematopoietic cell lines using this regimen before allogeneic BMT. In addition, the kinetics of hematopoietic reconstitution in our study appears similar to that of trials using TBI in conjunction with 120 mg/kg CY to prepare patients with AML prior to autologous BMT, which may also be indicative for the recovery from the transplanted marrow cells.

As can be expected with regimens leading to profound granulocytopenia, septicemic episodes were demonstrated in eight patients (40%) during marrow aplasia or early hematologic recovery. This figure equals the frequencies of septicemia after autologous BMT in patients with AML in first remission reported by others. One patient died during early marrow aplasia from an overwhelming septicaemia, and a second patient developed fatal bronchopneumonia despite absolute neutrophil counts beyond 1 × 10^{9}/L. It has to be emphasized that procedural fatal complications resulted exclusively from infectious causes that could not directly be related to the speed of hematopoietic reconstitution or the toxicity of the preparative regimen.

There are several limitations to a meaningful interpretation of the results regarding therapeutic efficacy in most uncontrolled trials of high-dose regimens necessitating autologous BMT as consolidation therapy for patients in first remission of AML. These are conditioned by patient exclusions due to early relapse, inappropriate patient numbers, a short follow-up, heterogeneity in terms of the preceding induction and postinduction chemotherapy, and the patient populations under study. In light of these general restrictions, the present study precludes valid conclusions regarding therapeutic efficacy in comparison to conventional chemotherapy protocols or other high-dose regimens with autologous BMT. Yet two potentially important and encouraging observations have to be stressed in this analysis. First, in contrast to the experiences with the preponderant proportion of chemotherapeutic trials, there is a clear-cut plateau phenomenon of the projected event-free survival curve, and the median of this curve has not been reached at 3 years after BMT. In considering that no relapse occurred beyond 11 years.
months from BMT, it appears reasonable to assume that the pattern of relapse is different from that seen with conventional maintenance therapy. Second, this analysis included four patients who did not enter complete remission after initial intensive induction therapy, and such patients are known to have an increased risk of leukemic relapse. All four patients remain in unmaintained remission at 36, 25, 20, and 15 months post-BMT and at 41, 38, 24, and 20 months after achieving complete remission. We strongly feel that these prolonged remissions can at least in part be attributed to the high-dose consolidation therapy.

The results achieved with this new preparative regimen in recipients of autografts in first remission of AML are promising in view of its satisfactory tolerability and the remarkably low rate of severe, nonhematotoxic toxic effects.

In particular, severe and life-threatening hepatic toxicity, a problem of the combination of high-dose busulfan with the higher dose of CY, had not been encountered. The event-free survival estimate observed in this analysis provides a basis for a wider application of this new preparative regimen and challenges comparisons with preparative regimens that include TBI as well as other chemotherapeutic regimens.

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REFERENCES

Acute toxicity and first clinical results of intensive postinduction therapy using a modified busulfan and cyclophosphamide regimen with autologous bone marrow rescue in first remission of acute myeloid leukemia [see comments]

DW Beelen, K Quabeck, U Graeven, HG Sayer, HK Mahmoud and UW Schaefer