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

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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 allogeneic 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).

INTENSIVE COMBINATION chemotherapy as primary treatment of acute myeloid leukemia (AML) leads to complete remission in a preponderant proportion of patients. However, despite various strategies to maintain remission, most patients relapse within the first 2 years after initial therapy, reflecting resistance of residual tumor cells to conventional doses of consolidation or maintenance chemotherapy. In patients with a histocompatible sibling donor, high-dose radiochemotherapy followed by allogeneic bone marrow transplantation (BMT) is presently regarded as the most effective treatment modality to consolidate first remission. As a preparative regimen for allogeneic BMT, the majority of transplant centers have employed a single or a daily fractionated dose of total body irradiation (TBI) in a dose range between 5 and 15.75 Gy, preceded or followed by 120 mg/kg cyclophosphamide (CY) given on two consecutive days. Using this combination, long-term remissions and possibly cure of the disease can be achieved in about 50% of patients with AML in first remission. Although encouraging, these results remain unsatisfactory in consideration of relapse rates of up to 25%, making recurrence of leukemia still a prominent cause of treatment failure in these patients.

The alkylating agent busulfan produces profound marrow aplasia in animal models and has shown a potent antileukemic and myeloablative effect in combination with CY. A combination of high-dose busulfan (16 mg/kg) and 200 mg/kg CY had first been used at the Johns Hopkins Oncology Center to prepare patients with AML for allogeneic BMT in 1975. An uncontrolled trial of this combination including patients with AML in first remission suggested a significantly lower relapse rate after allogeneic BMT, as can be achieved by regimens of TBI in conjunction with the widespread lower dose of 120 mg/kg CY. As a consequence of a relatively high rate of treatment-related complications, however, the overall disease-free survival in this study was similar when compared to regimens employing TBI. A high antileukemic efficacy of this combination was further supported by a study in 23 patients who received high-dose busulfan and CY followed by autologous BMT as consolidation therapy of AML in second or third remission. In this study marrow cells obtained during remission were incubated with the alkylating agent 4-hydroperoxy-cyclophosphamide intended to reduce the burden of contaminating clonogenic leukemic cells in the marrow suspension. The actuarial relapse rate of 46% at more than 50 months after autologous BMT appeared not inferior to that obtained with allogeneic or syngeneic BMT. Thus given a complete efficacy of marrow "purging," this regimen allowed disease control in about 50% of patients who seldom have durable remissions under conventional therapy. A recent study in allograft recipients using high-dose busulfan in combination with 120 mg/kg CY confirmed the excellent antitumor effect of this combination. In addition, a very low incidence of transplant-associated complications was described with this new preparative regimen, which might have resulted from the reduction of CY to 120 mg/kg.

In light of the reported, impressive tumoricidal effects, we initiated a study of intensive consolidation chemotherapy with high-dose busulfan in conjunction with CY and autologous marrow rescue in patients with AML in first remission who were not eligible for allogeneic BMT. For two reasons we decided to use the new regimen of 120 mg/kg CY in combination with 16 mg/kg busulfan. First, the serious toxicities described with the higher dose of CY were of...
special concern in a therapeutic approach, the significance of which has still to be established.3,4 Second, in contrast to allogeneic BMT, intensive immunosuppression, which pre-
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

Patient accrual 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 granulocyte-
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/m²/ d ARA-C as continuous infusion, day I and day 2,
30-minute infusions every 12 hours, days 3 through 8; 60 mg/m²/d
DNR intravenously [IV] days 3, 4, and 5; 100 mg/m² TG orally
every 12 hours, days 3 through 9) and further induction chemother-
apy.9 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/m² IV every 12 hours for three days) and mitoxantrone (10
mg/m²/d IV, days 3 through 5) according to the HAM protocol.11
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 of 10% of mye-
blasts 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 240); or one course of HAM followed by a combination of
ARA-C, etoposide, and amrascine (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/m² subcutaneously
[SC] every 12 hours, days 1 through 5) in combination with TG (200
mg/m²/d orally, days 1 through 5), DNR (45 mg/m²/d IV, days 3
and 4) or CY (1,000 mg/m² 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 char-
acteristics 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) × 10⁶ 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 red 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) × 10⁵ cells/kg body weight were recovered after separa-
tion. 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 cell concentration constantly exceeded 5 × 10⁸
cells/mL. One hundred thirty-milliliter aliquots were transferred to
polyolefin 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 10-21, 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 × 10⁵ marrow mononuclear cells separated by
Ficoll-Hypaque density 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
centrifugation. Colonies of greater than 50 cells were scored after 14
days incubation at 37°C, 10% CO₂, 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 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
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...given until a self-sustaining platelet count over 20 x 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 x 10⁹/L and the patients were afebrile and without signs of infection.

**Toxicity and infectious complications.** Drug-related toxic effects during the post-transplant period were evaluated by chart review and classified according to the graded World Health Organization toxicity scale. Diagnosis of infections was based on clinical findings together with cultural prove or serologic evidence of the infectious organism.

**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 an absolute neutrophil count over 1.0 x 10⁹/L and 5.0 x 10⁹/L or a platelet count in excess of 20 x 10⁹/L and 50 x 10⁹/L, respectively. Besides correlation analysis, a time-dependent, stepwise, proportional-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...
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 septicemia with Streptococcus sanguis septicemia 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 10⁹/L was 30 (range 20 to 53) days. A neutrophil count in excess of 1.0 x 10⁹/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 10⁹/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 10⁹/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 10⁹/L were reached between 11 and 28 (median 25) 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 marrow 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

<table>
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<th>CFU-GM Infused (&gt;0.5)</th>
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<th>Time to Relapse After ABMT</th>
<th>Remission Duration</th>
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Abbreviations: ANC, absolute neutrophil count; ABMT, autologous BMT; a/w, alive and well.
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Fig 1. Cumulative probability of reaching the endpoints of peripheral blood cell recovery: granulocyte counts >0.5 x 10^9/L (-----), granulocyte counts >1.0 x 10^9/L (----), platelet counts >20.0 x 10^9/L (------), platelet counts >50.0 x 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 ± 11%) 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 inexpedient 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 |
|-----------------------------|-----------|-----------|-----------|-----------|-----------|
| Grade 0                     | Grade 1   | Grade 2   | Grade 3   | Grade 4   |
| **Liver**                   | ≤1.25 x N*| 1.26-2.5 x N| 2.6-5 x N| 5.1-10 x N| >10 x N   |
| **Bilirubin**               | 7/20†     | 11/20     | 2/20      | -         | -         |
| **SGOT/SGPT**              | 3/20      | 10/20     | 4/20      | 3/20      | -         |
| **Renal**                   | ≤1.25 x N*| 1.26-2.5 x N| 2.6-5 x N| 5.1-10 x N| >10 x N   |
| **BUN**                    | 20/20     | -         | -         | -         | -         |
| **Creatinine**             | 20/20     | -         | -         | -         | -         |
| **Bladder**                | -         | -         | -         | -         | -         |
| **Hematuria**              | 17/20     | -         | 3/20      | -         | -         |
| **GI**                     | -         | -         | -         | -         | -         |
| **Oral**                   | 2/20      | 7/20      | 8/20      | 3/20      | -         |
| **Nausea/vomiting**        | -         | -         | 1/20      | 19/20     | -         |
| **Diarrhea**               | 13/20     | 7/20      | -         | -         | -         |
| **Cutaneous**              | 0/20      | 17/20     | 3/20      | -         | -         |
| **Neurotoxicity**          | -         | -         | -         | -         | 2/20      |

**State of consciousness**

- 17/20
- 1/20
- -
- -
- 2/20

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

autologous BMT using "purged" marrow.7,18 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 heterogenous group of patients using the higher dose of CY with busulfan 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

![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.](image_url)
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-cyclophosphamide, 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 LD100 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 myeloablative 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 septicemia, and a second patient developed fatal bronchopneumonia despite absolute neutrophil counts beyond 1 x 10⁹/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
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 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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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]

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