Autologous Bone Marrow Transplantation for Acute Myeloid Leukemia Using Monoclonal Antibody-Purged Bone Marrow


We report our experience from a clinical trial of autologous bone marrow transplantation (ABMT) in the treatment of 30 patients with acute myeloid leukemia (AML) using monoclonal antibody (MoAb) and complement-treated bone marrow. All patients were in complete remission (CR) at the time of transplant: 6 patients were in first CR, 18 in second CR, and 6 in third CR. The median age of all patients was 42 years (range 11 to 87 years). For marrow ablation, 28 patients were treated with cyclophosphamide and total body irradiation. One patient was treated with busulfan and cyclophosphamide and one was treated with busulfan and VP-16. Each patient was then transfused with autologous bone marrow that had been harvested previously and treated with two MoAbs, PM-81 and AML-2-23, and rabbit complement. Median time to recovery of neutrophils (500/µL) was 30 days, and platelets (20,000/µL) was 45 days. Median time for initial erythrocyte engraftment, assessed by a flow cytometric reticulocyte assay, was 13 days. Median overall and relapse-free survival of first CR patients was at least 17.4 months post-ABMT and the 2- and 3-year actuarial overall and relapse-free survival was 67% (± 19%). Median survival for the 24 patients in second or third CR was 6.8 months post-ABMT and 9.3 months since CR; however, six patients survived disease-free from 16 to 61 months post-ABMT. For the second and third CR group it was observed that six patients (5 of the 6 survivors) showed "inversions," when their post-ABMT remission lasted longer than any previous one. Actuarial 2- and 3-year disease-free and overall survival of patients in second and third CR was 26% (± 9%) and 18% (± 9%), and 29% (± 9%) and 23% (± 9%), respectively. ABMT avoids the problems of graft-versus-host disease and of finding suitable donors for allogeneic marrow transplantation.

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Acute myeloid leukemia (AML) afflicts approximately 11,000 new patients a year in the United States. Though treatment of AML with combination chemotherapy can induce a complete remission (CR) in 50% to 80% of patients, most patients subsequently relapse and ultimately succumb to their disease. Bone marrow transplantation (BMT) after high-dose chemotherapy and/or radiation therapy offers significant promise of complete elimination of occult leukemia cells during CR, and there is general agreement that BMT is probably the only curative treatment for patients with AML after first relapse. Encouraging results have been reported with allogeneic BMT, using bone marrow from HLA-identical or syngeneic donors as consolidation therapy in first CR. In second and third CR, allogeneic BMT can also be curative, although relapse-free survival in this setting is lower than in first CR, presumably due to increased chemotherapy resistance in previously treated patients. However, the majority of patients with AML either do not have an HLA-matched donor or are considered too old (over 45 years) for an allogeneic BMT. These people can be treated with autologous BMT (ABMT), which also avoids the complication of graft-versus-host disease (GVHD). To increase the efficacy of this treatment, methods of purging autologous marrow using monoclonal antibodies (MoAbs) or cytotoxic drugs are being evaluated.

We recently described a panel of cytotoxic MoAbs that react specifically with myeloid cells and recognize antigens expressed on AML blast cells. Of this panel the MoAbs PM-81 and AML-2-23 are the most reactive, binding with leukemia cells from greater than 95% of AML patients. In the presence of complement (C'), these MoAbs are cytotoxic to cells bearing the respective cell surface antigen(s), so they can lyse leukemia cells from almost all patients with AML, including their progenitor cells. Other studies showed that PM-81 and AML-2-23 do not recognize antigens on pluripotent stem cells, which are required for engraftment of transplanted marrow.

As of December 31, 1988 we have harvested, MoAb-purged, and transplanted bone marrow into 30 patients who were in CR at the time of transplant. Seven of these patients were described in a previous report. This paper updates our experience with this new approach to ABMT in AML.

Materials and Methods

Patients

Patients under the age of 65 years with good performance status were eligible for this protocol. Evaluations of cardiac, pulmonary, renal, and hepatic function were performed as detailed in each clinical protocol. Leukemia blast cells obtained at diagnosis or at relapse, when available, were required to express the antigens reactive with PM-81 and/or AML-2-23 on greater than 20% of cells. All patients were entered on protocols approved by the Institutional Review Boards of the respective institutions and gave informed consent for the treatment.

Immunophenotyping

Leukemia cells isolated by Ficoll-Hypaque gradient centrifugation were first incubated with human immunoglobulin G (IgG)
(10^-1 mol/L) (Sigma Chemical Company, St Louis, MO) to block binding through the Fc portion of the MoAb. Cells (10^6) were then incubated with saturating concentrations of MoAb, washed with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.1% sodium azide (AZ), and further incubated with fluorescein isothiocyanate (FITC)-coupled, affinity-purified F(AB')2 goat anti-mouse antibody (GAM) IgG (Boehringer Mannheim, Indianapolis, IN) for 30 minutes at 4°C. Cells were then washed once in PBS/BSA/AZ and resuspended in PBS/BSA/AZ containing 1% paraformaldehyde (Sigma). The cells were analyzed within 1 week on the Ortho Systems 50H flow cytometer (Ortho Diagnostics, Westwood, MA) interfaced to a 2150 computer using a linear amplification scale.

Marrow Harvesting and Purging

Bone marrow was harvested from the posterior and anterior iliac crests under general anesthesia and processed through a graded series of stainless steel filters. An effort was made to harvest 6 x 10^9 cells/kg body weight. A mean of 7.28 x 10^9 cells/kg were actually harvested. Postperfusion, there was a mean recovery of 20.6% of the cells, which were then treated. The aim was to treat 4 x 10^9 cells/kg with MoAb and freeze the remaining cells as a back-up. A mean of 9.97 x 10^9 cells/kg were treated, and from that there was a mean recovery of 39%. An average of 3.60 x 10^9 cells/kg was used for the transplant.

Bone marrow mononuclear cells were prepared first by buffy coat concentration and then Ficoll-Hypaque gradient centrifugation on the HemaSystems automated cell processor (HemaSystems Corp., Braintree, MA), a novel approach we pioneered in vitro manipulation of marrow cells with MoAbs, exposing them to a constant infusion of complement. Saturating amounts of purified MoAbs were preincubated with these cells for 15 minutes at a cell density of 10^7/mL followed by the addition of rabbit serum, achieving a final dilution of 1:6 (rabbit serum :medium). Dosage was determined in units of each MoAb. A unit was defined as the amount of MoAb that yielded 50% saturation of 10^9 target cells. To ensure saturation of all antigenic sites, the amount of each MoAb used was 10 U per 10^6 cells. Treatment on the HemaSystems cell processor was performed for 1 hour with continuous exposure to fresh complement, with simultaneous removal of spent complement, while centrifuging at room temperature. The MoAb treatment was performed in the presence of the enzyme deoxyribonuclease (DNAase) (10 U/mL) to reduce cell clumping. This treatment was performed on the HemaSystems cell processor for patients treated after May 1987 at the Dartmouth-Hitchcock Medical Center (DHMC, Hanover, NH). Before that date at the DHMC, and at the Scripps Clinic (La Jolla, CA) and Children's Hospitals (San Diego, CA), the marrow cells were treated in plastic or Teflon vessels (Savillex, Menlo Park, CA) and freeze the remaining cells as a back-up. A mean of 9.97 x 10^9 cells/kg were treated, and from that there was a mean recovery of 39%. An average of 3.60 x 10^9 cells/kg was used for the transplant.

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Colony-Forming Assays

Bone marrow mononuclear cells (2 x 10^5 cells) from pre- and post-treatment samples were cultured in quadruplicate in alpha medium (1 mL) containing 0.8% methylcellulose and 2-mercaptoethanol (2 x 10^-2 mol/L) as described. Erythropoietin (1 U/mL) (Connaught Laboratories, Swiftwater, PA) and giant cell tumor conditioned medium (GCT-CM) or recombinant granulocyte-monocyte colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) (generously provided by Dr Steven Gillis of Immunex Corporation, Seattle, WA) were added as sources of growth factors. BFU-E, CFU-GM, and mixed lineage colonies (CFU-MIX) of greater than 40 cells/aggregate were scored using an inverted microscope after 14 days in a 37°C, 5% CO2, atmosphere. Representative colonies were plucked from methylcellulose using a Pasteur pipet and Wright's-Giemsa-stained cytocentrifuge preparations were made to confirm cell lineage.

Preparative Regimen

Twenty-eight patients were treated with the following preparative regimen: Cyclophosphamide (CY) (60 mg/kg x 2 days) (days -5 to -3) and fractionated total body irradiation (TBI) (200 cGy twice daily for 3 days, total dose of 1,200 cGy) (days -2 to 0). One patient (in second CR) was treated with the following regimen: Busulfan (16 mg/kg/d for 4 days) (days -9 to -6) and cyclophosphamide (50 mg/kg/d for 4 days) (days -5 to -2). One patient (in third CR) was treated with the following regimen: Busulfan (16 mg/kg/d for 4 days) (days -7 to -4) and VP-16 (60 mg/kg) (day -3).

Cyclophosphamide and VP-16 were administered over 1 hour through a central venous catheter. Bladder irrigation was used to prevent hemorrhagic cystitis. Busulfan was administered orally. TBI was administered with a cobalt source at a dose rate of 5 to 10 cGy/min as previously described. All patients underwent lumbar puncture within 1 week before ABMT, at which time 12 mg of methotrexate was instilled. After ABMT, most patients received an additional two doses of I.V. methotrexate.

Statistical Analysis

Actuarial lifetables and survival curves for overall and relapse-free survival were computed separately for first and later remission patients, both since last CR and ABMT. The Cox proportional hazards model was used to evaluate factors associated with overall and relapse-free survival, post-CR, and ABMT in second/third remission patients only, since there were too few first CR patients to perform this analysis. The factors examined were the percent recovery of total bone marrow mononuclear cells and of CFU-GM, BFU-E, and CFU-MIX, days to achieve engraftment of neutrophils, and French-American-British (FAB) subclass at diagnosis.

The effect of the MoAb and C' treatment on CFUs was evaluated as the median percent recovery of absolute number of CFU in pretreatment samples. This number was derived by dividing the absolute numbers of CFU in posttreatment samples by the number of CFU contained in the pretreatment samples. Medians and Spearman rank correlations were reported rather than means and Pearson correlations because the distribution of the percent recovery values was very skewed.

RESULTS

Patients

Thirty patients with AML ranging in age from 11 to 53 years who were in CR were transplanted between August
1984 and December 31, 1988 (Table 1). All but one patient had de novo AML at the time of initial diagnosis. This patient had a myelodysplastic syndrome before diagnosis of AML. Three patients were transplanted at the Scripps Clinic, one patient at Children's Hospital, San Diego, and 26 patients at the DHMC. With two exceptions, the patients met the specified criteria for cardiac, pulmonary, renal function, and hepatic function. One patient had elevated hepatic enzymes, presumably due to non-A, non-B hepatitis. One patient had an abnormal left ventricular ejection fraction. The FAB subclasses of the cases were as follows: M1, 3; M2, 10; M3, 6; M4, 9; M5, 2. The median time between the current remission and ABMT was 60 days, with a range of 4 days to 15 months. The transplants in the six patients in first CR were performed at a median time of 10 months (range 6 to 14 months) after achieving CR.

Data on cell surface antigen expression were available on 24 patients. All cases were greater than 20% positive for PM-81. On average, 78.5% of leukemia cells were positive for binding to MoAb PM-81 (range 31% to 100%, median 81%). Nine of 24 cases were positive for binding to MoAb AML-2-23. Seven of these nine were M4 or M5 FAB; one was M2 and one M3.

Toxicity

Preparative regimen. The preparative regimen was generally well-tolerated. Most patients experienced mild to moderate nausea and vomiting during the administration of chemotherapy and TBI. Mucositis was moderate to severe. Diarrhea was experienced by the majority patients in the first 2 weeks after TBI. All but two patients became febrile during the period of marrow hypoplasia and leukopenia and required multiple parenteral antibiotics, including amphotericin B.

Four patients died within 2 months of ABMT while still in the recovery phase. One died from overwhelming fungal sepsis, one from veno-occlusive disease of the liver (the patient with the elevated hepatic enzymes), one from hemorrhagic complications due to refractoriness to platelet transfusions, and one from cardiopulmonary failure. Three additional patients died from nonleukemic causes related to ABMT. Two died at 3 and 8 months from intracerebral hemorrhages while still thrombocytopenic. Another patient died of overwhelming pseudomonal sepsis at 3 months while his bone marrow was still markedly hypocellular. All of the patients who died of nonleukemia related causes were in either second or third CR at the time of ABMT.

Unusual complications included a Coomb's positive autoimmune hemolytic anemia (AIHA) in one patient and a Legionella soft-tissue abscess in another. The patient with AIHA developed bilateral aseptic necrosis of the femoral heads 1 year post-ABMT, probably related to the use of high-dose corticosteroids to treat the hemolysis.

Marrow infusion. The infusion of bone marrow was well-tolerated. All patients were premedicated with diphenhydramine, hydrocortisone, and acetaminophen. Several patients developed myalgias and urticaria responsive to antihistamines. A change in the marrow purging protocol, which included more extensive washing of the final cell product, resulted in the elimination of such reactions.

Five patients required a second infusion of MoAb-treated marrow when engraftment appeared to be delayed. In each case, a moderately severe reaction occurred. In one patient this was manifest as hypotension associated with syncope. In the other patients, respiratory distress associated with pulmonary infiltrates developed several hours after the infusion. Each patient was treated with aggressive fluid and corticosteroid therapy and all reactions were reversed without sequelae. None of the patients required intubation and mechanical ventilation. In each case engraftment followed the infusion of the treated "back-up" bone marrow. None of the patients with prolonged thrombocytopenia received "back-up" marrow.

CFUs

The effect of the MoAb and C' treatment on CFUs was determined by culture of cells in methylcellulose. The median recovery of CFU-GM progenitor cells was 36% (range 22 to 150) for the first CR group and 47% (range 17 to 156) for the second/third CR group (Table 2). Median recovery of BFU-E was 38% (range 34 to 93) for the first CR group and 68% (range 13 to 1,003) for the second/third CR group. Median recovery of CFU-MIX was 46% (range 29 to 96) for the first CR group and 37% (range 0 to 226) for the second/third CR group. Median numbers of 1.02 x 10^4 and 0.8 x 10^4 CFU-GM/kg body weight were infused in each first and second/third CR patient, respectively, at the time of ABMT.

Engraftment

A median number of 2.5 x 10^7 cells/kg body weight (range 1.7 to 8.2 x 10^7) were infused into each first CR patient. The median number of cells transfused into the second/third CR group was 2.8 x 10^7/kg (range 0.8 to 7.4 x 10^7).

### Table 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Remission</th>
<th>N</th>
<th>Ages (median)</th>
<th>Male:Female</th>
<th>FAB Subclass</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>6</td>
<td>34-46 (43)</td>
<td>4:2</td>
<td>M1/M2 3 2 1</td>
</tr>
<tr>
<td>Second</td>
<td>18</td>
<td>11-53 (38)</td>
<td>11:7</td>
<td>M3        7 3 8</td>
</tr>
<tr>
<td>Third</td>
<td>6</td>
<td>29-57 (44)</td>
<td>5:1</td>
<td>M4/M5 3 1 2</td>
</tr>
<tr>
<td>All</td>
<td>30</td>
<td>11-57 (42)</td>
<td>20:10</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Effect of MoAb and C' Treatment on CFUs

<table>
<thead>
<tr>
<th>Remission</th>
<th>N*</th>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>CFU-MIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>3</td>
<td>36 (22-150)</td>
<td>38 (34-93)</td>
<td>46 (29-96)</td>
</tr>
<tr>
<td>Second/third</td>
<td>19</td>
<td>47 (17-156)</td>
<td>68 (13-1,003)</td>
<td>37 (0-226)</td>
</tr>
</tbody>
</table>

*Number of patients whose CFUs were studied and evaluable.
†Percentage of CFUs remaining after marrow purging (see text for calculation).
‡Sample size was 15 for CFU-MIX.
Engraftment of granulocyte, monocyte, and erythrocyte precursors was prompt in most patients (Table 3). Only one patient in third CR, who was extensively pretreated, failed to engraft and died 3 months posttransplant from sepsis related to neutropenia. Median recovery times for neutrophils to 500 cells/μL were 27 and 32 days for the first and second/third CR patients, respectively. Erythrocyte engraftment, assessed by a flow cytometric reticulocyte maturation assay, was 13 days (range 8 to 27) for 14 evaluable patients (two first CR, 12 second or third CR). Median times to reach platelet counts of greater than 20,000 and greater than 50,000 μL were 38 and 66 days (first CR) and 46 and 82 days (second/third CR).

Days to achieve engraftment was negatively correlated with weight-adjusted CFU-GM, BFU-E, CFU-MIX, and the number of cells infused (Table 4), when all patients were analyzed together. This means engraftment was faster in those infused with larger numbers of CFU.

**Relapse**

Two first CR patients relapsed, one at 10 months and one at 16 months post-ABMT. At the time of ABMT the CR1 durations of the patients that relapsed were 12 and 7 months, respectively. The pre-ABMT CR durations of the other four first CR patients now surviving relapse-free after the transplant were 6, 9, 11, and 14 months. Ten second/third CR patients relapsed at times ranging from 3 to 29 months post-ABMT. Median time to relapse for second/third CR patients was 11.4 months post-ABMT and 19.5 months post-CR. Of the patients transplanted in the second and third CR, six were shown to have inversions where the duration of their post-AMBT CR2 or CR3 exceeded the duration of the CR1 or CR2 by 2, 8, 11, 28, and 29 months. Five of these are in the group of six survivors.

In all cases studied, the morphologic and immunologic phenotypes of the leukemia cells obtained at relapse were similar to those of the original sample obtained at diagnosis or the relapse before transplant. The leukemia cell morphology (at diagnosis) of patients who relapsed was FAB M1 or M2 in six and FAB M4 or M5 in the other six.

One second CR patient who relapsed 2 years after first ABMT was successfully reinduced into a third CR, and underwent a second ABMT using busulfan and cyclophosphamide as the preparative regimen and MoAb-purged bone marrow. He successfully engrafted and survives disease-free 39 months after second ABMT. The data from the second ABMT were not included in the analysis.

**Survival**

The survival, relapse-free survival, and relapse rate from transplant of all patients as of October 1, 1989 are shown in Figs 1, 2, and 3 by CR group. The median relapse-free survival time of the CR1 group has not been reached, since half of first CR patients had not relapsed as of October 1, 1989; however, it is at least 17.4 months post-ABMT and 24 months post-CR. Actuarial 2-year survival and relapse-free survival post-ABMT are both 67% (±19%). Four of the six patients are surviving at 23, 25, 35, and 41 months post-ABMT.

Actuarial 2- and 3-year survival of the patients in the second/third CR group is 29% (±9%) and 23% (±9%), respectively, from last remission and the same from the time of ABMT. Median survival of this group is 6.8 months from the date of ABMT and 9.3 months from the documentation of the current remission; median relapse-free survival post-ABMT is 5.2 months. Six patients survive disease-free at 16, 20, 32, 41, 55, and 61 months post-ABMT.

Of the patients transplanted in the second and third CR, six were shown to have inversions where the duration of their post-AMBT CR2 or CR3 exceeded the duration of the CR1 or CR2 by 2, 8, 11, 28, and 29 months. Five of these are in the group of six survivors.

Both the FAB subclass and the percent recovery of BFU-E after marrow purging were related to relapse-free survival in the second/third CR group. The risk of relapse or death increased by 2.7% for every 10% increase in BFU-E recovery ($P = .05$). This small effect on survival is probably not significant clinically. Patients with FAB subclasses M4 and M5 had better disease-free survival than other subclasses. Their rate of mortality or relapse was on average 15% (95% confidence interval, 3% to 85%; $P = .03$) that of the other subclasses. Although the 2-year survival rates were comparable, FAB M1, M2, and M3 patients as a group demonstrated a higher rate of early relapse or mortality. These results were similar for overall and relapse-free survival post-CR. Al-

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### Table 3. Times to Achievement of Engraftment Milestones in Patients Undergoing ABMT

<table>
<thead>
<tr>
<th>CR</th>
<th>N†</th>
<th>PMN &gt; 500/μL</th>
<th>Hgb &gt; 10g%</th>
<th>Plt &gt; 20,000/μL</th>
<th>Plt &gt; 50,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>5</td>
<td>27 (15-79)</td>
<td>50 (15-222)</td>
<td>38 (17-83)</td>
<td>66 (21-229)</td>
</tr>
<tr>
<td>Second/third</td>
<td>18</td>
<td>32 (12-212)</td>
<td>61 (6-454)</td>
<td>46 (22-251)</td>
<td>82 (28-333)</td>
</tr>
<tr>
<td>All patients</td>
<td>23</td>
<td>30</td>
<td>57</td>
<td>45</td>
<td>82</td>
</tr>
</tbody>
</table>

*Independent of transfusions of erythrocytes or platelets.
†Data are unavailable for six patients in second/third CR because of early deaths.
‡Range of days to achieve engraftment.

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### Table 4. Correlation Coefficients Between Engraftment and CFU Data

<table>
<thead>
<tr>
<th>Days to Achieve</th>
<th>No. Cells Infused*</th>
<th>CFU-GM*</th>
<th>BFU-E*</th>
<th>CFU-MIX*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN &gt; 500/μL</td>
<td>-0.23</td>
<td>-0.80‡</td>
<td>-0.60‡</td>
<td>-0.49§</td>
</tr>
<tr>
<td>Platelets &gt; 20,000/μL</td>
<td>-0.64‡</td>
<td>-0.46</td>
<td>-0.69‡</td>
<td>-0.55‡</td>
</tr>
<tr>
<td>Hgb &gt; 10 g%</td>
<td>-0.50§</td>
<td>-0.79‡</td>
<td>-0.67‡</td>
<td>-0.21</td>
</tr>
<tr>
<td>RMI increase‡</td>
<td>-0.65§</td>
<td>-0.65§</td>
<td>-0.31</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

*Per kilogram body weight.
†Time at which a rise was noted in the reticulocyte maturation index (RMI) post-ABMT.
‡$P < .01$.
§$P < .05$.
||$.05 < P < .01$. 

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Fig 1. Overall survival of 30 patients who have undergone ABMT with MoAb- and C'-purged bone marrow. Solid line represents patients in first CR (six patients) and the dashed line represents patients in second and third CR combined (24 patients).

though not formally analyzed due to small sample size, other factors associated with mortality in this study included a history of refractoriness to platelet transfusions and abnormal liver and cardiac function. Three deaths in the immediate posttransplant period were directly related to these antecedent problems.

DISCUSSION

Considerable activity in the use of ABMT has been reported over the past few years. Most centers in the US use a purging technique, usually involving a cytotoxic drug or MoAbs, to eradicate residual occult disease from the autologous marrow. A number of multicenter, uncontrolled, European studies demonstrated that AML patients transplanted within the first 6 months of their first CR show a relapse-free survival benefit using marrow that had been treated with mafosfamide (a congener of 4-hydroperoxycyclophosphamide, 4-HC) rather than unpurged marrow. Though no randomized studies directly comparing ABMT with and without marrow purging have been reported from any center for any group of patients, long-term survival has been noted for AML patients in second and third CR after ABMT using various methods for removing occult leukemia cells. Because most studies of ABMT without marrow purging in both previously relapsed patients and in first CR patients have shown a very high relapse rate, these findings suggest the need for marrow purging.

Overall, the results of the present study are encouraging, though we urge caution in interpreting our results in first CR patients because it is impossible to know what proportion of this small group were previously cured by chemotherapy. However, the results for patients in second and third CR can be assessed with more confidence, and here a median survival of 9.3 months post-CR is superior to the results of investiga-
tional chemotherapeutic agents,\(^1\) and similar to allogeneic BMT for patients at similar risk for relapse\(^{2-7}\) as well as to alternative approaches to ABMT in AML, such as the use of 4-HC to treat bone marrow.\(^{15,34}\) Our study demonstrated 3-year overall survival in approximately 23% of patients in second and third CR and relapse-free survival of 18%. These calculations do not include the data from the second transplant in the patient who was transplanted twice, and thus slightly underestimate the relapse-free survival figure. Median time to relapse, censoring nonleukemic deaths, was 11.4 months, computed using actuarial methods. Interestingly, there was a survival benefit in this study for patients with FAB M4, M5 subclasses. This contrasts with many studies that have shown that FAB M4 and M5 have had worse survival rate.\(^5,6,7,8\) Whether this finding can be attributed to marrow purging with MoAb to myeloid antigens will require further study.

The updated Johns Hopkins experience with 4-HC purged marrow transplantation in second and third CR shows that disease-free survival was achieved in 34% and 28% of patients, respectively, with a median time to relapse of 10.4 months.\(^{34}\) However, caution should be observed when comparing the results of purging methods with patients receiving different ablative regimes. The Johns Hopkins study used busulfan and cyclophosphamide as preparative treatment, and while there is no randomized, direct comparison between cyclophosphamide/busulfan versus cyclophosphamide/TBI for ablation in AML, it appears that there is a higher relapse rate in patients receiving the latter regimen.\(^{4,9}\) When used in the allogeneic BMT of patients in second and third CR, the busulfan/cyclophosphamide regimen was associated with relapse in only 1 of 17 patients (6% relapse rate),\(^2\) while cyclophosphamide and TBI resulted in 28% 5-year disease-free survival, with relapse rates of 37 to 47%.\(^4,8\) The patients in our study were also older, with a median age of 42 years compared with 31 years in the Hopkins study, a factor that probably contributed to the morbidity and mortality of our procedure.

Four of six patients transplanted in first CR are disease-free at times ranging from 20 to 38 months post-ABMT (67% overall and relapse-free survival at 2 years) and 25 to 52 months from achievement of first CR. Procedure-related toxicity in this group of patients was not severe and there were no treatment-related deaths. Five of 10 patients in first CR transplanted in the Johns Hopkins study are disease-free with a probability of 43% disease-free survival at 2 years.\(^{34}\) Allogeneic BMT in first CR can achieve 46% five-year disease-free survival.\(^4\) Since both allogeneic and autologous BMT are associated with better long-term disease-free survival when applied to patients in first CR\(^4,5,16,17,22,33\) compared with later remissions, we are developing a study comparing ABMT with conventional therapy in patients in first CR.

Using our median time for platelet recovery as about 2 months (see Table 3), eight of the evaluable patients were slow to recover platelets. This almost certainly contributed to the demise of two patients, neither of which had a second marrow infusion. Interestingly, every patient with prolonged thrombocytopenia that we examined had evidence of mega-karyocyte engraftment in a day 28 post-ABMT bone marrow biopsy. It should be noted that most studies of ABMT for AML with or without marrow purging also have reported prolonged thrombocytopenia in some patients, thus suggesting a problem independent of MoAb therapy.\(^31,32\) It is unclear what allows some patients to survive for long periods of time with slowly increasing platelet counts while other patients develop bleeding diathesis that leads to death. After 45 days, patients who were engrafting slowly and had the back-up marrow available were reinfused. Though initially it was thought that this second infusion should use untreated marrow, the patients chose to have MoAb purging performed before reinfusion. Complete engraftment followed the reinfu-
In each of the five cases. It is difficult to assess whether the success of these engraftments was due to the second infusion or a slow response to the initial transplant. Only one patient appeared to suffer from a failure of engraftment, dying 3 months posttransplant of pancytopenia. These results seem to demonstrate the equivalent safety of antibody-complement purging with other ABMT procedures.

If complement-mediated purging is used to treat remission bone marrow of AML patients, MoAbs PM-81 and AML-2-23 appear to be ideally suited to identify the leukemia cells for several reasons. First, of more than 350 AML samples analyzed, 91% and 77% of patients reacted with PM-81 and AML-2-23, respectively. Leukemia progenitors from greater than 80% of patients expressed CD15 the antigen defined by PM-81, while 40% expressed the CD14 antigen defined by AML-2-23. Because of the heterogeneity of tumors it is important to use combinations of MoAbs since together they will bind to a greater percentage of cells from a given population of leukemia cells than either one alone. Second, both MoAbs are cytotoxic in the presence of rabbit C'. Third, the MoAbs detect antigens expressed at the level of the normal CFU-GM, but not at the level of the pluripotent hematopoietic stem cell. Thus, they react with "early" hematopoietic antigens, an advantage for removal of leukemia cell progenitors while sparing pluripotent progenitors necessary for engraftment. Fourth, we demonstrated the ability of the combined MoAbs to kill six to seven logs of hematopoietic stem cell. Thus, they react with "early" hematopoietic antigens, an advantage for removal of leukemia cell progenitors while sparing pluripotent progenitors necessary for engraftment. Therefore, we demonstrated the ability of the combined MoAbs to kill six to seven logs of clonogenic leukemia cells.

The rate of trilineage engraftment appears to be influenced by the absolute numbers of CFU infused. In light of the observation that total cell number infused did not correlate as strongly with neutrophil engraftment as the number of CFU, it might be possible to improve the kinetics of engraftment by augmenting the numbers of CFU for patients in whom the CFU numbers were low at initial bone marrow harvest. This could be accomplished by harvesting bone marrow or by the use of peripheral blood stem cells. The slow return of platelets in some patients may be related to the modest reduction in CFU. The time to reach more than 20,000 platelets/μL did correlate inversely with the numbers of CFU-GM, BFU-E, and CFU-MIX. Similarly, the time to achieve reticulocytosis, stable red blood cell levels, and red blood cell transfusion independence correlated inversely with the numbers of CFU-GM and BFU-E infused.

Based on this pilot study, the Cancer and Leukemia Group B (CALGB) has activated two ABMT protocols for AML that use PM-81 and AML-2-23 to purge the bone marrow. One protocol is for patients with AML in second and third CR, while the other is for patients at first relapse (who will have had marrow harvested, treated, and stored during remission). The rationale for the latter study is the observation of the Seattle group that long-term survival is similar in patients treated with allogeneic BMT at first relapse or in second remission. Performing transplants at relapse as remission-inducing therapy could allow a greater salvage rate since less than 50% of patients achieve second CR with remission induction therapy in most studies. We anticipate that these studies will provide further supportive data for the wider use of ABMT for patients with AML.

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REFERENCES

human bone marrow transplantation: Ricin linked to three monoclonal antibodies. Science 222:512, 1985


30. Dicke KA, Spitzer G: Evaluation of the use of high-dose cytoereduction with autologous marrow rescue in various malignancies. Transplantation 41:4, 1986


