Autologous Bone Marrow Transplantation in High-Risk Remission B-Lineage Acute Lymphoblastic Leukemia Using a Cocktail of Three Monoclonal Antibodies (BA-1/CD24, BA-2/CD9, and BA-3/CD10) Plus Complement and 4-Hydroperoxycyclophosphamide for Ex Vivo Bone Marrow Purging

By Fatih M. Uckun, John H. Kersey, Robert Haake, Daniel Weisdorf, and Norma K.C. Ramsay

Fourteen patients with high-risk B-lineage acute lymphoblastic leukemia (ALL) in complete remission underwent autologous bone marrow transplantation (BMT) using a combined immunocompromising protocol. A monoclonal antibody (MoAb) cocktail of BA-1, BA-2, and BA-3 plus rabbit complement (C') plus 4-hydroperoxycyclophosphamide (4-HC) was used to eliminate residual occult leukemia cells from autografts. All patients were conditioned with single-dose total body irradiation (TBI) followed by high-dose Ara-C. All 14 patients engrafted at a median of 24 days (range, 12 to 36 days). Three patients are alive and disease free at 3.5 years, 3.9 years, and 4.1 years post-BMT. The Kaplan-Meier estimate and standard error of the probability of sustained remission was 23% ± 12% at 3.5 years post-BMT with a mean relapse-free interval of 1.4 ± 0.4 years. The disease-free survival (DFS) at 3.5 years was 21% ± 11%, with a mean DFS time of 1.3 ± 0.4 years. A novel and quantitative minimal residual disease (MRD) detection assay, which combines fluorescence-activated multiparameter flow cytometry and cell sorting with leukemic progenitor cell (LPC) colony assays, was used to analyze remission BM samples from B-lineage ALL patients for residual LPC, and to evaluate the efficacy of ex vivo BM purging. Notably, the minimal residual leukemia burden before BMT, as measured by the percentage of B-lineage LPC in the pre-BMT remission BM samples, indicated the outcome of the BMT. The median value for the minimal residual leukemia burden before BMT was 0.0035% (35 LPC/10^6 mononuclear cells). The Kaplan-Meier estimates and standard errors of the probability of remaining in remission after BMT were 43% ± 19% for patients whose BM samples contained ≤0.0035% LPC and 0% ± 0% for patients whose BM samples contained greater than 0.0035% B-lineage LPC (P < .05). In contrast to the minimal residual leukemia burden measured by the described MRD assay system, the percentage of blasts or TdT+ cells in the remission BM samples did not correlate with the probability of relapse. The applied purging protocol showed variable success in destroying target B-lineage LPC populations contaminating the autografts. While in some cases purging was highly effective, eliminating up to >4 logs of residual B-lineage LPC in other cases only 0.1 to 0.2 logs of B-lineage LPC were purged. Because of this variation in efficacy, the estimated numbers of B-lineage LPC remaining in autografts, as well as the estimated numbers of reinfused LPC, showed a marked interpatient variation. The investigators conclude that improvements in both pretransplant conditioning and ex vivo BM purging may be needed to significantly increase the percentage of long-term survivors among high-risk B-lineage ALL patients undergoing autologous BMT.

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and postpurge samples of clinical autografts were analyzed for the presence of B-lineage LPC. This report details our laboratory and clinical findings using the minimal residual disease (MRD) assay system with 14 high-risk B-lineage ALL patients who underwent autologous BMT during complete remission.

MATERIALS AND METHODS

Patients. Fourteen patients with high-risk B-lineage ALL in complete remission were enrolled in a clinical study of autologous BMT. The study protocol was approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota, and written informed consent was obtained from all patients according to Department of Health and Human Services guidelines. To be eligible for this protocol, patients had to have leukemic blasts that were immunophenotyped at diagnosis or relapse and found positive for CD9, CD10, or CD24 B-lineage-associated differentiation antigens. Fourteen patients were enrolled in this study. There were two females and 12 males with a median age of 13 years (mean ± SE = 14 ± 3 years; range, 4 to 36 years). The initial white blood cell (WBC) count ranged from 1,200 to 1,290,000/μL (median, 12,650; mean ± SE = 113,343 ± 90,802). The surface antigen profile of leukemic blasts was determined in all patients at the time of diagnosis and/or relapse before BMT. Six of seven cases (100%) expressed CD9, 14 of 14 cases (100%) expressed CD10, and 8 of 8 cases (100%) expressed CD24. One patient was in first remission (UPN 815, an adult male with Philadelphia chromosome [Ph]-positive ALL), seven were in second remission, five were in third remission, and one patient was in fourth remission. Except for UPN 815, all patients had prior BM relapses and five patients had prior extramedullary relapses as well. Of these five patients, three had central nervous system (CNS) relapses and two had testicular relapses. In the 13 patients who were in their second, third, or fourth remission, the duration of their first remissions had ranged from 6 months to 62 months (mean ± SE = 30 ± 5 months; median, 31 months).

BM harvest. BM was harvested under general anesthesia by multiple aspirations from the iliac crests in volumes to yield a minimum of 4.5 × 10^9 nucleated cells/kg. The BM was processed and treated ex vivo with a combination of three MoAb + C' and 4-HC, as described below. Nucleated cells (8.5 to 1.0 × 10^9) were stored untreated as backup BM to be used in case of graft failure.

Ex vivo purging of autologous BM grafts. The treatment of BM mononuclear cells (MNC) from remission autologous BM grafts with BA-1, BA-2, BA-3 MoAb plus C' in combination with 4-HC for elimination of residual leukemic blasts has been described in detail elsewhere. Detection of residual leukemic blasts in autologous BM grafts before and after purging. Ficoll-Hypaque-separated BM MNC from pre- and postpurge samples of autologous BM grafts were analyzed by two-color immunofluorescence for the "early" B-lineage cell surface differentiation antigen CD19 using phycoerythrin (PE)-labeled B43 (anti-CD19) MoAb, and the "late" B-lineage cell surface differentiation antigen sIgM using fluorescein isothiocyanate (FITC)-conjugated 2C3 (anti-IgM) MoAb, as described. Sterile cell sorting at 1,500 cells/s was performed to isolate viable CD19(red) sIgM (green) B-cell precursors (BCP) in the "lymphoid" and "blast" light scattering windows, as described. Fluorescence-activated cell sorting (FACS) was performed and sorted BCP were assayed for B-lineage ALL blast colony formation in the presence of 10% vol/vol low molecular weight B-cell growth factor (L-BCGF; Cellular Products, Buffalo, NY) in a LPC assay system, as detailed in previous publications. After 7 days of culture, whole petri dishes containing the cultures were harvested for morphologic and immunologic characterization of colony cells, as previously described. In addition to this MRD detection assay, we also used multicolor multiparameter flow cytometry (FCM) without cell sorting to monitor the lysis of target B-lineage lymphoid cell populations by the MoAb + C' component of the purging protocol. To this end, BM MNC from pre- and postpurge samples of autologous BM grafts were analyzed in a comparative fashion by two-color immunofluorescence for the presence of CD10(J5-PE')CD19(B43-FITC') + , CD10(J5-PE')CD22(G28-7-FITC') + , and CD19(B43-PE')sIgM(2C3-FITC') - BCP within the lymphoid light scattering window, as described.

Preparative regimens and supportive care. All 14 patients were prepared with single-dose TBI (850 cGy total dose delivered using 10-MeV X-rays at a dose rate of 26 cGy/min on day −8) followed by 12 doses of Ara-C on days −7 through −2 at a dose of 3 g/m² administered as a 1-hour intravenous infusion twice daily (total dose of Ara-C, 36 g/m²). After 1 day of rest (day −1), autologous BM grafts were thawed and administered on day 0. The details of this preparative regimen were previously published. Supportive care during the hospital stay was provided as previously detailed.

Data analysis. The clinical and laboratory data were retrieved from the University of Minnesota BMT Data Base, which systematically and prospectively collects data on all of our BM transplant recipients. The data were analyzed by standard statistical methods with the assistance of the BMDP-90 software program (University of California Press, Berkeley, CA). Posttransplant event times were measured from the day of BMT to the day of relapse, death, or last follow-up. Disease-free survival (DFS) was defined as the time from date of BMT to date of relapse or death, whichever occurred first. The probabilities for remaining in remission and DFS were estimated and relapse-free interval curves were generated using the Kaplan-Meier product limit method. We used the Cox regression method to examine the relationship between the five potential predictor variables (%TdT + cells, % lymphoblasts, % B-lineage LPC in the prepuge remission BM samples, LPC/kg reinfused in the autografts, and the log kill of LPC by the applied purging protocol) and relapse post-BMT. Given the wide ranges in values for the percent LPC in the prepuge BM samples and for LPC per kilogram, log transforms were applied to the above listed potential predictors. Due to the number of statistical tests performed, Bonferroni adjustments were made to the resulting P values. Statistical significance is quoted only for those predictors that remain significant (P < 0.05) after adjustment for multiple comparisons.

RESULTS

Quantitative analysis of BM remission status by multiparameter FCM, cell sorting, and LPC colony assays. The percentage of lymphoblasts in the pretransplant BM specimens ranged from 0% to 2.7% (mean ± SE = 0.9% ± 0.2%; median, 0.6%) and the percentage of TdT + cells ranged from 0% to 4.5% (mean ± SE = 1.3% ± 0.4%; median, 1.0%) (Table 1). In an attempt to more accurately examine the BM remission status of patients, we performed a quantitative analysis of residual leukemia in remission BM specimens. Specifically, we analyzed the CD19 sIgM BCP fraction within the "lymphoid" plus "blast" light scattering windows for the presence of B-lineage LPC. The percentage of CD19 sIgM BCP among the BM MNC showed a marked interpatient variation ranging from 0.2% (UPN 198...
0749) to 39.8% (UPN 0661) (mean ± SE = 7.2% ± 2.7%; median, 5.0%). When stimulated with L-BCGF (10% vol/vol), FACS-sorted virtually pure populations of CD19+sIgM- cells from 14 of 14 patients formed 4 to 523 (mean ± SE = 164 ± 39; median, 145) blast colonies per 100,000 cells (Table 1). Colony cells displayed blast morphology with a high nuclear to cytoplasmic ratio, highly irregular nuclear membrane, and prominent nucleoli (Fig 1). In nine cases, sufficient numbers of colony cells were obtained for immunophenotypic analyses. As shown in Table 2, colony blasts were TdT+ (7 of 7 cases; mean ± positivity, 90% ± 3%), CD10/CALLA+ (8 of 9 cases; mean ± positivity, 68% ± 9%), CD19+ (9 of 9 cases; mean ± positivity, 93% ± 3%), cytoplasmic CD22+ (4 of 4 cases; mean ± positivity, 99% ± 1%), CD24+ (7 of 7 cases; mean ± positivity, 76% ± 5%), and slg- (8 of 8 cases; mean ± positivity, 1% ± 0.5%), consistent with B-lineage ALL. In two cases (UPN 0661 and UPN 0771) colony blasts expressed cytoplasmic μ heavy chains (Cy), consistent with pre-B ALL, while the remaining seven cases were Cy- negative (mean ± positivity, 2% ± 1%), consistent with pre–pre-B ALL. The number of B-lineage LPC per 10⁶ MNC in the analyzed remission BM specimens from all 14 patients was calculated using the formula: B-lineage LPC/10⁶ MNC = [CD19+sIgM- MNC (%)/100] × [mean no. of blast colonies/10⁵ FACS-sorted cells] × [10⁵/10⁶]. Controls included normal BM specimens from four healthy volunteers and leukemic BM specimens from five newly diagnosed B-lineage ALL patients.

**Abbreviation:** ND, not determined.

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**Table 1. Residual Leukemia in High-Risk Remission B-Lineage ALL BM Samples**

<table>
<thead>
<tr>
<th>UPN</th>
<th>BM Remission No.</th>
<th>% Lymphoblasts</th>
<th>% TdT+</th>
<th>% CD19+sIgM- BCPs</th>
<th>Mean No. of Blast Colonies per 10⁵ FACS-Sorted BCPs</th>
<th>Immunophenotypic Features of Colony Blasts in L-BCGF-Stimulated Cultures of FACS-Sorted CD19+sIgM- BCPs</th>
<th>B-Lineage LPC per 10⁶ MNC</th>
<th>B-Lineage LPC % of MNC</th>
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<td>0.0</td>
<td>0.0</td>
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<td>523</td>
<td>85 69 98 100 85 3 2</td>
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<td>0.0</td>
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<td>44</td>
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**Normal BM controls**

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<th>% Lymphoblasts</th>
<th>% TdT+</th>
<th>% CD19+sIgM- BCPs</th>
<th>Mean No. of Blast Colonies per 10⁵ FACS-Sorted BCPs</th>
<th>Immunophenotypic Features of Colony Blasts in L-BCGF-Stimulated Cultures of FACS-Sorted CD19+sIgM- BCPs</th>
<th>B-Lineage LPC per 10⁶ MNC</th>
<th>B-Lineage LPC % of MNC</th>
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**B-lineage ALL BM controls**

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<th>% Lymphoblasts</th>
<th>% TdT+</th>
<th>% CD19+sIgM- BCPs</th>
<th>Mean No. of Blast Colonies per 10⁵ FACS-Sorted BCPs</th>
<th>Immunophenotypic Features of Colony Blasts in L-BCGF-Stimulated Cultures of FACS-Sorted CD19+sIgM- BCPs</th>
<th>B-Lineage LPC per 10⁶ MNC</th>
<th>B-Lineage LPC % of MNC</th>
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<td>BO</td>
<td>(90% blasts)</td>
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<td>1,682</td>
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<td>JZ</td>
<td>(90% blasts)</td>
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<tr>
<td>AS</td>
<td>(100% blasts)</td>
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<td>490</td>
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<td>JC</td>
<td>(95% blasts)</td>
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<td>207</td>
<td>ND 80 95 ND 95 ND 2</td>
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<tr>
<td>CH</td>
<td>(95% blasts)</td>
<td>95</td>
<td>930</td>
<td>ND 85 100 ND 0 ND 0</td>
<td>8,835.0 0.88</td>
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**Fresh BM samples obtained at the time of BM harvest in complete remission were examined for the presence of residual LPC, as described in Materials and Methods. Colony assays were set up in duplicate. The variation in the number of TdT+CD19+ blast colonies from individual dishes did not exceed 30% of the mean values. The immunophenotypic features of colony blasts were determined as described in Materials and Methods, and the phenotype data are shown as the percentage of colony blasts expressing each marker. The numbers of B-lineage LPC per 10⁶ MNC were calculated from the percentage of FACS-sorted BCP among MNC and the numbers of TdT+CD19+ blast colonies using the formula: B-lineage LPC/10⁶ MNC = [CD19+sIgM- MNC (%)/100] × [mean no. of blast colonies/10⁵ FACS-sorted cells] × [10⁵/10⁶]. Controls included normal BM specimens from four healthy volunteers and leukemic BM specimens from five newly diagnosed B-lineage ALL patients.**

**Fig 1. Detection of residual CD19+ LPC in remission BM samples of high-risk B-lineage ALL patients.** (A) In situ morphology of day 7 blast colonies in L-BCGF-stimulated cultures of FACS-sorted CD19+sIgM- BCP from pre-purge autograft samples of two high-risk remission B-lineage ALL patients. (B) Wright-Giemsa-stained preparations of colony blasts from day 7 blast colonies shown in (A). Note the immature blast morphology with irregular nuclei, prominent nucleoli, and high nuclear/cytoplasmic ratio. (C) Nuclear TdT staining of day 7 colony blasts shown in (B). (D) Surface CD19 antigen expression of day 7 colony blasts shown in (B) and (C).
Fig 1.
proliferation and colony formation of normal BCP from fetal hematopoietic organs.\textsuperscript{15} Notably, unlike normal BCP from fetal hematopoietic organs,\textsuperscript{15} FACS-sorted CD19'sIgM- BCP from B-lineage ALL remission BM specimens did not differentiate during the 7-day culture period in the presence of L-BCGF. Although FACS-sorted BCP from B-lineage ALL remission BM did not display any blast colony formation in L-BCGF-stimulated cultures of FACS-sorted CD19'sIgM- BCP, the fetal hematopoietic organs. Notably, unlike normal BCP phenotype data are shown as the percentage of colony blasts expressing each marker.

### Table 2. Efficacy of Purging Against Residual B-lineage LPC in High-Risk Remission B-lineage ALL Autografts

<table>
<thead>
<tr>
<th>UPN</th>
<th>% CD19'sIgM- BCP Among MNC</th>
<th>Mean No. of Blast Colonies/10^5 MNC</th>
<th>Immunophenotypic Features of Colony Blasts in L-BCGF-Stimulated Cultures of FACS-Sorted CD19'sIgM- BCP (% cells positive)</th>
<th>B-Lineage LPC per 10^6 MNC</th>
<th>% B-Lineage LPC Among MNC</th>
<th>Log Kill</th>
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<tr>
<td>0648</td>
<td>0.25</td>
<td>104</td>
<td>78</td>
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<td>0661</td>
<td>0.69</td>
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<td>95 95 0 74 85 ND</td>
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<td>0666</td>
<td>0.30</td>
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<td>≤0.000003</td>
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<td>0723</td>
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<td>&lt;0.01</td>
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Samples from purged autografts were analyzed for the presence of residual B-lineage LPC as described in Materials and Methods. The numbers of B-lineage LPC were calculated as described in the legend of Table 1. Log kill values were obtained by comparing on a logarithmic scale the number of B-lineage LPC per 10^6 MNC in pre purge autograft samples shown in Table 1 with the number of B-lineage LPC per 10^6 MNC in post purge autograft samples shown in this table. The immunophenotypic features of colony blasts were determined as described in Materials and Methods, and the phenotype data are shown as the percentage of colony blasts expressing each marker.

Abbreviation: ND, not determined.

### Efficacy of ex vivo BM purging

As illustrated by the representative cases shown in Fig 2, the purging protocol had variable success in destroying CD19'CD10', CD19'CD1D', CD19'sIgM', or CD10'CD22' BCP populations within the lymphoid light scattering window. Comparison of the pre purge and post purge autograft specimens indicated a prompt and nearly complete elimination of BCP from post purge autograft samples. When the residual CD19'sIgM- BCP from post purge autograft samples were isolated by FACS sorting decreased because of the higher percentage of nonviable LPC contamination (data not shown). Thus, results obtained using remission BM from B-lineage ALL patients were not obscured by normal autologous BCP populations capable of clonogenic growth in the presence of L-BCGF.
Fig 2. Multiparameter FCM analysis of elimination of BCP populations from autologous BM grafts by immediate lytic effects of the combined immunochemopurging protocol. MNC from prepurge and postpurge autograft samples were stained with various MoAb combinations and analyzed for the presence of CD19" IgM", CD10"CD19", CD10" CD19", CD10" CD22" BCP populations. Note in (A) the effective elimination of BCP from the autograft of UPN 0661 by purging and, in sharp contrast to it, in (B) the failure of the purging protocol to eliminate BCP from the autograft of UPN 0837.

2, colony blasts were TdT" (4 of 4 cases, mean % positivity = 86% ± 6%), CD10" (5 of 5 cases, mean % positivity = 81% ± 9%), CD19" (5 of 5 cases, mean % positivity = 88% ± 2%), cytoplasmic CD22" (2 of 2 cases), CD24" (5 of 5 cases, mean % positivity = 79% ± 7%), and IgM" (4 of 4 cases, mean % positivity = 2% ± 2%), consistent with B-lineage ALL. In two cases (UPN 0661 and 0771), colony blasts were Cp" consistent with pre-B ALL. Thus, the immunophenotypic features of colony blasts in the cultures of BCP from postpurge autograft samples were identical to those of colony blasts in the cultures of BCP from prepurge samples. In particular, blasts did not differ in expression of CD10 (prepurge, 68 ± 9% vs. postpurge 81% ± 9%, P = .4) or CD24 (prepurge 76% ± 5% vs. postpurge 79% ± 7%, P = .8), two of the three antigens targeted by the MoAb cocktail. This finding rules out lack of expression of these antigens as the underlying cause of inefficient purging. The estimated numbers of B-lineage LPC in the postpurge autografts ranged from less than 0.01 LPC/10^6 MNC (ie, <0.0000006% of MNC) in UPN 0755 (Table 2) (mean ± SE = 24.8 ± 16.2 LPC/10^6 MNC; median, 0.250 LPC/10^6 MNC (ie, mean ± SE = 0.0025% ± 0.0016%; median, 0.000025%). The comparison of the B-lineage LPC numbers in the prepurge autograft samples (Table 1) versus postpurge samples (Table 2) showed a marked interpatient variation in the antileukemic efficacy of the applied BM purging protocol. In one case (UPN 0723), B-lineage LPC numbers in prepurge and postpurge samples were not sufficient to estimate the log kill efficacy of purging. In six cases, purging was highly effective, eliminating ≥2.48 logs to >4.28 logs of residual B-lineage LPC, and in five cases it was moderately effective, eliminating 1.12 logs to 2.13 logs of residual B-lineage LPC. The mean log kill efficacy in these 11 cases was 2.4 ± 0.3 logs. In the remaining two cases (UPN 0755 and UPN 0837), purging was not effective at all, and only 0.13 to 0.20 logs of B-lineage LPC were eliminated.

BMT and posttransplant course. The post-BMT events are detailed in Table 3. The cell dose of the BM grafts...
Table 3. BMT and Posttransplant Course

<table>
<thead>
<tr>
<th>Marrow Graft</th>
<th>Time to Engraftment (d)</th>
<th>Time to Event (d)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN</td>
<td>Cell Dose (&lt;10^9/kg)</td>
<td>LPC/kg</td>
<td>Relapse</td>
</tr>
<tr>
<td>0648</td>
<td>0.72</td>
<td>190</td>
<td>24</td>
</tr>
<tr>
<td>0661</td>
<td>0.57</td>
<td>75</td>
<td>29</td>
</tr>
<tr>
<td>0666</td>
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<td>20</td>
</tr>
<tr>
<td>0689</td>
<td>0.72</td>
<td>&lt;7</td>
<td>29</td>
</tr>
<tr>
<td>0723</td>
<td>0.52</td>
<td>&lt;2</td>
<td>24</td>
</tr>
<tr>
<td>0749</td>
<td>0.57</td>
<td>&lt;1</td>
<td>36</td>
</tr>
<tr>
<td>0755</td>
<td>0.99</td>
<td>20.592</td>
<td>19</td>
</tr>
<tr>
<td>0770</td>
<td>0.83</td>
<td>&lt;1</td>
<td>17</td>
</tr>
<tr>
<td>0771</td>
<td>0.32</td>
<td>293</td>
<td>36</td>
</tr>
<tr>
<td>0814</td>
<td>0.54</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>0815</td>
<td>0.70</td>
<td>42</td>
<td>28</td>
</tr>
<tr>
<td>0837</td>
<td>0.86</td>
<td>9,766</td>
<td>12</td>
</tr>
<tr>
<td>0850</td>
<td>0.87</td>
<td>&lt;1</td>
<td>21</td>
</tr>
</tbody>
</table>

The preparative regimen, supportive care, method of BM processing, and BMT were described in previous reports. *Abbreviation: GI hemorrhage, gastrointestinal hemorrhage.*

The estimated numbers of reinfused B-lineage LPC (LPC/kg = reinfused cell dose/kg \times \%LPC among MNC of post-purge autograft samples/100) ranged from less than 1 LPC/kg in UPN 0671, UPN 749, UPN 0770, and UPN 850, to 20,592 in UPN 0755 (mean ± SE = 2,214 ± 1,574; median, 13/kg). All 14 patients engrafted, as defined by a WBC greater than 1,000/µL for 3 consecutive days, at a median of 24 days (range, 12 to 36 days; mean ± SE = 24 ± 2 days). One patient died on day 72 of hemorrhage and respiratory failure due to adult type respiratory distress syndrome (ARDS). Ten patients relapsed at 73 to 731 days (mean ± SE = 233 ± 62 days; median, 182 days) post-BMT and eight have subsequently died of leukemia. Of the remaining two patients who relapsed, UPN 689 died of interstitial pneumonitis on day 544 and UPN 815 died of Aspergillus pneumonia on day 418. Three patients (UPN 0723, 0749, and 0814) are alive and disease free at 3.5 years, 3.9 years, and 4.1 years post-BMT. The Kaplan-Meier estimate and standard error of the probability of remaining in remission was 23% ± 12% at 3.5 years post-BMT with a mean relapse-free interval of 1.4 ± 0.4 years (median, 0.6 years) (Fig 3A). The overall DFS at 3.5 years was 21% ± 11% with a mean DFS time of 1.3 ± 0.4 years (median, 0.5 years).

**Correlation between MRD and probability of relapse post-BMT.** Cox regression was used to determine the relationships between relapse-free interval and the five laboratory parameters of MRD. After making Bonferroni adjustments for multiple comparisons, the percent B-lineage LPC in the pre-BMT/prepurge remission BM samples was a significant predictor of relapse (P < .05). The product-limit estimates for the probability of sustained remission among patients with BM LPC percentages less than or equal to versus greater than the median value of 0.0035% are depicted in Fig 3B. The Kaplan-Meier estimates and standard errors of the probability of remaining in remission after BMT were ranged from 0.32 \times 10^9/kg to 0.99 \times 10^9/kg (mean ± SE = 0.70 ± 0.05 \times 10^9/kg; median, 0.73 \times 10^9/kg).
43% ± 19% for patients whose BM samples contained 
≤0.0035% B-lineage LPC and 0% ± 0% for patients whose 
BM samples contained greater than 0.0035% B-lineage 
LPC. LPC per kilogram was also a significant predictor of 
relapse post-BMT (P < 0.05). The Kaplan-Meier estimates 
and standard errors of the probability of remaining in 
remission after BMT were 33% ± 19% for patients who 
received less than the median estimated value of 13 LPC/kg 
in their reinfused autografts and 14% ± 13% for patients 
who received ≥13 LPC/kg in their reinfused autografts 
(data not shown). No significant relationships were found 
between the probability of remaining in remission and the 
percentage of TdT+ cells, percentage of lymphoblasts, or 
log kill of LPC contaminating the autografts by the purging 
protocol (all P > .05).

**DISCUSSION**

We used a new BM purging protocol that combines a 
cocktail of BA-1/anti-CD24, BA-2/anti-CD9, and BA-3/ 
anti-CD10, MoAb, rabbit C', and 4-HC, in an attempt to 
imimize the chance of reinfusing significant numbers of 
leukemic blasts in purged autografts. All patients showed 
prompt engraftment at a median of 24 days, which provides 
evidence that this immunochemopurging protocol does not 
significantly impair the repopulating capacity of autografts. 
Three patients remain alive and disease free at 3.5 years, 
3.9 years, and 4.1 years post-BMT, which shows that 
prolonged DFS can be achieved in some high-risk remission 
B-lineage ALL patients who do not have a matched sibling 
donor by autologous BMT during complete remission using 
ex vivo purged autografts.

In recent years, several laboratories have developed 
highly sensitive MRD detection methods to discern very 
small numbers of residual leukemic blasts in remission BM 
samples from acute and chronic leukemia patients. Proposed strategies include multiparameter FCM and im-
munophenotyping, cytogenetic, biophysical, and biochemical 
analyses, clonogenic assays, Southern blot hybridization 
analyses of Ig or T-cell receptor gene rearrangements, and 
polymerase chain reaction (PCR) technology for amplifica-
tion of DNA sequences flanking the crossover sites of 
clonotypic chromosomal translocations or rearranged T-cell 
receptor γ- or δ-chain sequences. 

Hansen-Hagge et al reported that the PCR amplification of rearranged T-cell 
receptor δ-chain sequences using clonospecific probes per-
mits the detection of as little as 0.0001% leukemic cell 
DNA. Similar levels of sensitivity in detecting residual 
ALL blasts were reported by d’Auriol et al, who used PCR 
amplification of T-cell receptor γ-chain DNA sequences, 
by Yamada et al, who used PCR amplification of comple-
mentary-determining region III DNA sequences, as 
well as by Kawasaki et al and Hunger et al, 
who used PCR amplification of leukemia-specific messenger RNA (mRNA) 
sequences. In more recent studies, several investigators 
have started to evaluate the clinical value of PCR-based 
MRD detection assays. Yamada et al presented 
molecular evidence for the persistence of residual disease 
during the first 18 months of treatment in eight of eight 
children with B-lineage ALL. Intriguing data published 
recently by Neale et al suggested that the detection of MRD 
in T-lineage ALL by PCR technology may predict impending 
relapse. Gehly et al provided elegant evidence that 
PCR-detectable chimeric BCR-abl mRNA can be used as a 
marker for MRD for prediction and/or early identification 
of relapse in Philadelphia chromosome-positive ALL patients 
undergoing BMT. Yokota et al reported that detection 
of MRD by PCR predicted clinical relapse in two 
patients with ALL. Recently, we developed a novel 
quantitative MRD detection assay system for T-lineage 
ALL, which combines multiparameter FCM and FACS 
sorting with LPC assays. Importantly, high numbers of 
residual LPC in remission BM of high-risk T-lineage ALL 
patients before BMT appeared to constitute a poor prognos-
tic indicator, providing evidence for the clinical value of in 
vitro LPC colony assays in BMT.

Our published results on the use of multiparameter FCM 
and FACS sorting with LPC assays for detection of residual 
clonogenic blasts in remission BM from T-lineage ALL 
patients prompted us to design a similar MRD detection 
assay for B-lineage ALL. This new MRD assay for B-lin-
edge ALL is based on FACS sorting of CD19+slgM- BCP 
from B-lineage ALL remission marrow samples and their in 
vitro culture in the presence of L-BCGF using a B-lineage 
LPC colony assay system. Intriguingly, high numbers of 
residual B-lineage LPC in remission BM immediately 
before BMT predicted relapse, while the percentage of 
lymphoblasts and the percentage of TdT+ cells were not 
prognostic. We did not obtain sufficient numbers of cells 
from day 7 blast colonies to perform Southern blot hybrid-
ization or cytogenetic analyses to confirm the clonality and 
leukemic origin of colony blasts. Therefore, we cannot 
formally exclude the possibility that a small fraction of blast 
colonies counted as TdT+CD10+CD19+slgM- BCP 
colonies were derived from normal CD10+CD19+slgM- 
B-lineage lymphoid progenitor cells. However, (1) the correla-
tion of LPC numbers with the probability of relapse; (2) the lack of differentiation of FACS-sorted CD19+slgM- 
BCP from B-lineage ALL remission BM samples, in sharp 
contrast to the rapid differentiation (as measured by 
acquisition of C4a and slgM) of normal BCP from fetal 
hematopoietic organs; (3) the absence of detectable B-line-
age LPC in negative control BM specimens from healthy 
donors; (4) the presence of varying numbers of B-lineage 
LPC in all positive control BM specimens from B-lineage 
ALL patients in partial relapse or from newly diagnosed 
B-lineage ALL patients; and (5) the strong correlation 
between the number of viable leukemic blasts added to 
remission BM samples from B-lineage ALL patients and 
the numbers of LPC provide strong, albeit circumstantial, 
evidence that this MRD detection assay system measures 
the leukemic clonogenic cell burden of BM specimens, and 
that the results are not complicated by normal BCP 
populations capable of clonogenic growth in the presence 
of L-BCGF.

Our preliminary findings reported here indicate that the 
MRD detection assay system described in the present
report may have clinical value because it appears to reflect the total leukemia burden or total clonogenic ALL blast pool during remission more accurately than the conventional enumeration of lymphoblasts or TdT⁺ cells in BM. We therefore hypothesize that this assay system will help us to determine when the residual disease burden of high-risk ALL patients is sufficiently small to allow the eradication of occult leukemia cells by high-dose chemotherapy or radiochemotherapy before BMT. Separate long-term studies on a much larger patient population will be needed to determine whether the MRD data generated using this assay system will enhance the predictive strength of established prognostic parameters such as WBC at diagnosis. In comparison to cytogenetic or molecular genetic analyses, this MRD detection assay system does not require the presence of clonal chromosomal abnormalities or the availability of clonospecific probes, thereby providing an opportunity for routine analysis of MRD in remission ALL BM samples. Furthermore, a quantitative analysis of remission BM samples for the presence of residual clonogenic ALL blasts by this MRD assay system will likely yield more biologically relevant information about the quality of remission than qualitative analyses that do not discriminate between ALL blasts of different clonogenic or proliferative potential, such as PCR amplification of clonotypic DNA sequences. Perhaps the combined use of this MRD assay and a highly sensitive PCR method in future studies will yield the most reliable and biologically most significant information regarding the quality of remission in B-lineage ALL patients. Such “second generation” combined MRD assays would provide the opportunity (1) to compare the quality of remission achieved after different conditioning regimens in both allogeneic and autologous BMT; (2) to measure the effects of graft-versus-host disease or the regimens used to prevent graft-versus-host disease (such as cyclosporin A or methotrexate) on the residual leukemia burden; and, most importantly, (3) to compare the residual leukemia burden of long-term survivors of ALL who have undergone either multiagent chemotherapy or BMT.

Surprisingly, our BA-1,2,3 + C’ + 4-HC immunochemopurging protocol showed variable success in destroying target B-lineage LPC populations. While in some cases purging was highly effective, eliminating up to ≥4 logs of residual B-lineage LPC, in other cases purging eliminated only 0.1 to 0.2 logs of B-lineage LPC. Because of this inconsistency, the estimated numbers of remaining B-lineage LPC in postpurge autografts as well as the estimated numbers of reinfused LPC showed a marked interpatient variation. While several factors (eg, variations in antigen expression, C’ sensitivity, 4-HC sensitivity, or shielding of leukemic blasts by normal cell populations with the same target B-lineage differentiation antigens) may contribute to the variable efficacy of this combined protocol, multiparameter FCM analysis of the immediate lytic effects of BM purging on target BCP populations provided strong evidence for a marked heterogeneity among patients’ sensitivity to C’. The immunophenotypic analyses of target BCP populations and LPC-derived colony blasts have shown that the interpatient variation in the efficacy of this immunochemopurging protocol was not caused by marked qualitative or quantitative differences in expression of target antigens. Notably, patients who received higher numbers of LPC in their autografts had earlier and more frequent relapses, as compared with patients whose autografts were contaminated with no or very few remaining LPC after purging. Because higher numbers of LPC in prepurge autograft samples were associated with higher numbers of remaining LPC after purging, the observed relationship between LPC per kilogram and relapse could be explained by the predictive value of the LPC numbers in the prepurge autograft samples. Whether reinfused LPC contributed to the higher relapse rate in patients who had more LPC in their autografts needs to be investigated in a larger series of patients. Improvements in both pretransplant conditioning and ex vivo BM purging should increase the percentage of long-term survivors among high-risk B-lineage ALL patients undergoing autologous BMT.

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Autologous bone marrow transplantation in high-risk remission B-lineage acute lymphoblastic leukemia using a cocktail of three monoclonal antibodies (BA-1/CD24, BA-2/CD9, and BA-3/CD10) plus complement and 4-hydroperoxycyclophosphamide for ex vivo bone marrow purging

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