Residual Bone Marrow Leukemic Progenitor Cell Burden after Induction Chemotherapy in Pediatric Patients with Acute Lymphoblastic Leukemia

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ABSTRACT

We used highly sensitive multiparameter flow cytometry and blast colony assays to quantify the leukemic progenitor cell (LPC) burden of postinduction chemotherapy bone marrows from newly diagnosed and relapsed pediatric patients with acute lymphoblastic leukemia (ALL). Of 890 newly diagnosed patients, 243 (27%) had detectable LPC in the postinduction bone marrow samples with an average (mean ± SE) LPC content of 22 ± 9 LPC/10^6 mononuclear cell (MNC; range, 0–7199/10^6 MNC; median, 0/10^6 MNC). By comparison, 24 of 50 (48%) patients with relapsed ALL had detectable LPC in their postinduction bone marrow specimens (P = 0.003), and their average LPC content was 202 ± 139 LPC/10^6 MNC. Fewer patients with B-lineage ALL (170 of 786; 22%) than patients with T-lineage ALL (73 of 104; 70%) harbored residual LPC in their postinduction bone marrow specimens (P = 0.0001). This correlation with immunophenotype was independent of the National Cancer Institute risk classification. Similarly, 19 of 44 (43%) patients with relapsed B-lineage ALL versus 5 of 6 (83%) patients with relapsed T-lineage ALL harbored residual LPC in their postinduction bone marrow specimens (P = 0.09). Among newly diagnosed patients, those with high-risk ALL seemed to have larger numbers of residual LPC in their bone marrow after induction chemotherapy than those with standard risk ALL (53 ± 26, n = 286 versus 7 ± 1, n = 604, P = 0.04). LPC of patients with standard risk ALL who had a slow early marrow response at day 7 seemed to be more resistant to the three-drug induction chemotherapy than patients who had a rapid early marrow response. Overall, the order of chemosensitivity of LPC was: newly diagnosed standard risk B-lineage > newly diagnosed higher risk B-lineage > newly diagnosed standard risk T-lineage > newly diagnosed higher risk T-lineage > relapsed B-lineage > relapsed T-lineage. Notably, LPC^- patients whose end-of-induction remission bone marrow specimens had zero LPC had an excellent event-free survival outcome. Within the standard and high-risk subsets, LPC^- patients had a 2.6-fold lower and 2.4-fold lower incidence of events, respectively, than LPC^+ patients. At 6 months, 12 months, as well as 24 months, the ranking order for better event-free survival was: standard risk, LPC^- > high risk, LPC^- > standard risk, LPC^+ > high risk, and LPC^+.

INTRODUCTION

ALL is the most common form of childhood cancer (1–17). A major focus in contemporary translational ALL research is the evaluation of the biological significance of detection of small numbers of residual leukemic cells (i.e., MRD) in postchemotherapy remission bone marrow specimens (18–38). Numerous MRD detection methods are being tested for their ability to predict relapse (18–38). Because ALL is a curable disease responsive to several different treatment modalities, identification of patients who are at high risk for relapse may allow prevention of imminent relapse by altering therapy.

We have developed a quantitative test that combines multiparameter flow cytometry, cell sorting, and blast colony assays to discern small numbers of LPCs in the bone marrow of patients with ALL (39–42). This MRD assay allows routine analysis of MRD burden in bone marrow from all patients with ALL in remission, because it does not require the presence of clonal chromosomal abnormalities or probes specific to a particular leukemic clone (39–42). We previously used this method to compare the residual LPC burden in bone marrow specimens from 83 patients with ALL undergoing autologous BMT in remission (41). In these patients, the bone marrow LPC count varied markedly, ranging from 0–12,546 cells per million MNCs, or from 0–1,255% (median, 51 LPCs per million MNCs, or 0.005%). Patients whose LPC counts exceeded the median value had a higher likelihood of relapse than did patients with LPC counts below the median. The estimated relative risk

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3 The abbreviations used are: ALL, acute lymphoblastic leukemia; MRD, minimal residual leukemia; LPC, leukemic progenitor cell; BMT, bone marrow transplantation; EFS, event-free survival; CCG, Children’s Cancer Group; IT, intrathecal; RER, rapid early response; SER, slow early response; MNC, mononuclear cell; FACS, fluorescence-activated cell-sorting; NCI, National Cancer Institute.
of relapse for patients with \( \geq 51 \) LPCs per million MNCs was greater than 3.5 times the risk for patients with lower counts, after adjustment for the effects of other covariates (41). The inverse relationship we observed between the burden of LPC before BMT and the length of remission after BMT indicated that the determination of the LPC count in remission bone marrows could be clinically useful as a measure of the quality of remission and as a guide to treatment allocation. More recently, we used this method for evaluation of the bone marrow remission status of pediatric ALL patients with an isolated extramedullary relapse (42, 43).

Currently, very little is known about the LPC content of bone marrows in newly diagnosed ALL patients after induction chemotherapy. The purpose of the present study was to evaluate the quality of first remission in a large series of pediatric patients with ALL. We also sought to compare the quality of remission to that of second remission achieved in patients with an early first bone marrow relapse. Furthermore, we compared the in vivo chemotherapy sensitivity of LPC, as measured by log reduction of pretreatment bone marrow LPC content after induction chemotherapy, in relation to NCI risk classification, immunophenotype, day 7 bone marrow status, and disease status (i.e., initial diagnosis versus first relapse). We also examined the prognostic significance of the end-of-induction MRD status on early EFS outcome of newly diagnosed patients within the first 2 years of study entry. To our knowledge, this is the largest study, to date, that has examined the quality of first remission in childhood ALL.

**MATERIALS AND METHODS**

**Patients.** This analysis involves 940 children with ALL who were enrolled on CCG protocols for standard risk ALL (CCG-1952; \( n = 604 \)), higher risk ALL (CCG-1961; \( n = 286 \)), or ALL in first bone marrow relapse (CCG-1941; \( n = 50 \)) and achieved a complete remission after induction chemotherapy. Initial diagnosis of ALL was based on morphological, biochemical, and immunological features of the leukemic cells, including lymphoblast morphology on Wright-Giemsa-stained bone marrow smears, negative staining for myeloperoxidase, and cell surface expression of lymphoid differentiation antigens, as described previously (44–48). Patients were classified as B-lineage if \( \geq 30\% \) of the leukemic cells were positive by flow cytometry for CD19 and \( < 30\% \) were positive for one or more of the T cell-associated antigens CD2, CD3, CD5, or CD7. Likewise, patients were classified as T-lineage if \( \geq 30\% \) of the isolated blasts were positive by flow cytometry for one or more of the T cell-associated antigens CD2, CD3, CD5, or CD7 and \( < 30\% \) were positive for CD19. All protocols were approved by the NCI and the Institutional Review Boards of the participating CCG-affiliated institutions. Informed consent was obtained from parents, patients, or both, as deemed appropriate, according to Department of Health and Human Services guidelines.

**Treatment.** Patients with standard risk ALL as defined by NCI risk criteria (1–9 years of age with WBC \( < 50,000/\mu l \)) were enrolled on CCG-1952 beginning in May 1996 and received systemic therapy based on previous low and intermediate risk CCG ALL protocols. Induction therapy consisted of the three drugs: vincristine, prednisone, l-asparaginase, along with IT cytarabine and IT methotrexate. Higher-risk patients (\( \geq 10 \) years of age and/or with WBC \( \geq 50,000/\mu l \)) were enrolled on CCG-1961 beginning in September 1996 and received four-drug induction with vincristine, prednisone, l-asparaginase, and daunorubicin, along with IT cytarabine and IT methotrexate. Patients with an early first bone marrow relapse who relapsed either on treatment or within 12 months after completion of chemotherapy were enrolled on CCG-1941 beginning in March 1995 and received a 5-week reinduction with etoposide, ifosfamide, dex-amethasone, vincristine, l-asparaginase, i.v. methotrexate, and IT triple therapy consisting of methotrexate, hydrocortisone, and cytarabine.

**Day 7 Bone Marrow Morphological Response.** Patients on the CCG-1952 and CCG-1961 protocols were required to have bone marrow aspirates performed at day 7 (\( \pm 1 \) day) of induction chemotherapy to determine the status of early marrow response. The percentage of blasts present was determined at each patient’s local institution, based on 100 cell differentials whenever possible. RER was defined as \( \leq 25\% \) blasts; SER was defined as \( > 25\% \) blasts.

**Assay for Residual LPCs.** We used a quantitative assay system to detect residual leukemia in the bone marrow specimens from patients with ALL. The system combines multiparameter flow cytometry and cell sorting using a FACS Vantage Instrument (Becton Dickinson) with LPC colony assays to measure the residual burden of clonogenic blasts in the bone marrow. The FACS Vantage instrument is equipped with an air-cooled argon laser (488 nm at 50 mW). A detailed description of the assay system and its validated ability to detect residual clonogenic blasts in bone marrow samples from patients with ALL has been published previously (39–41). Briefly, CD7\(^+\)/CD3\(^-\) T-cell precursors were sorted at 1000–2000 cells/sec from Ficoll-Hypaque-purified bone marrow MNCs using two-color multiparameter flow cytometry with fluorescently labeled monoclonal antibodies directed against CD7 (3A1-RD1; catalogue no. 6603827; Coulter-Immunotech) and CD3 (catalogue no. IM1281; Coulter-Immunotech). The LPC fraction of these CD7\(^+\)/CD3\(^-\) precursors was quantified by counting the number of blast colonies that formed after 7 days of culture in conditions optimized for growth of T-lineage ALL cells (39, 43). Similarly, CD19\(^+\)/surface IgM\(^-\) B-cell precursors were sorted at 1000–2000 cells/sec from Ficoll-Hypaque-purified bone marrow MNCs using two-color multiparameter flow cytometry with fluorescently labeled monoclonal antibodies directed against CD19 and surface IgM (40). The anti-CD19 antibody was phycoerythrin-labeled B43 (41, 42), and the anti-sIgM antibody was goat antihuman IgM \( \mu \) chain (Biosource International, Camarillo, CA). The LPC fraction of these CD19\(^+\)/surface IgM\(^-\) precursors was quantified by counting the number of blast colonies that formed after 7 days of culture in conditions optimized for growth of B-lineage ALL cells (39, 41). We previously demonstrated that control bone marrow specimens from healthy volunteer donors contain no detectable LPCs under these assay conditions (39–41) and a strong correlation exists between the number of viable leukemic blasts added to remission bone marrow samples and the numbers of LPCs (39–41). Data were expressed as the number of LPCs per one million bone marrow MNCs. The log kill of LPC by induction chemotherapy was determined using the formula:
log \text{ kill} = \log \left[ \frac{\text{preinduction LPC burden}}{\text{postinduction LPC burden}} \right]. In 651 newly diagnosed and 26 relapsed patients who had no detectable LPCs in their postinduction bone marrow specimens, we used a value of 1 for the postinduction LPC burden to estimate the minimum log kill of LPCs.

### Statistical Methods.
Correlations of bone marrow LPC burden with other clinical and laboratory parameters were analyzed by standard statistical methods, including Student’s t test and χ² analysis. P < 0.05 was considered to be significant. A P between 0.05 and 0.1 was considered to be of borderline significance.

### RESULTS

#### Immunophenotypic Characteristics of Patients.
Seven hundred eighty-six of the 890 newly diagnosed patients (88%), including 568 (94%) of the standard risk patients and 218 (76%) of the high-risk ALL patients, had B-lineage ALL. The remaining 104 of the 890 newly diagnosed patients (12%) had T-lineage ALL. All 50 children in first bone marrow relapse had relapsed either on treatment or within 1 year after completion of chemotherapy and were treated according to the CCG-1941 salvage protocol. Of these 50 patients, 44 (88%) had B-lineage ALL and 6 (12%) had T-lineage ALL (Table 1).

### Preinduction Chemotherapy Bone Marrow LPC Burden.
As shown in Table 1, the pretreatment bone marrow LPC contents were 3433 ± 158/10⁶ MNC for newly diagnosed standard risk patients, 3021 ± 173/10⁶ MNC for newly diagnosed high-risk patients, and 2848 ± 529/10⁶ MNC for relapsed patients. Pretreatment LPC content was similar for newly diagnosed patients regardless of NCI risk classification or immunophenotype. Furthermore, patients with relapsed B-lineage ALL had LPC counts similar to those of the overall group of newly diagnosed B-lineage patients (3098 ± 590/10⁶ MNC versus 3287 ± 131/10⁶ MNC, P = 0.4). There were too few relapsed patients with T-lineage ALL (n = 6) to allow meaningful statistical comparisons with other groups, although this subset seemed to have fewer LPC (1017 ± 343/10⁶ MNC) in the preinduction bone marrow than any other patient subpopulation.

### Postinduction Chemotherapy Bone Marrow LPC Burden.
Among the standard risk subset, 155 (26%) patients had detectable LPC in their end-of-induction bone marrows, with a mean (±SE) of 7 ± 1 LPC/10⁶ MNC and a median of 0 LPC/10⁶ MNC (range, 0–330 LPC/10⁶ MNC; Table 2). Among high-risk patients, 88 (31%) had detectable LPC in their end-of-induction bone marrows, with a mean (±SE) of 53 ± 26 LPC/10⁶ MNC and a median of 0 LPC/10⁶ MNC (range, 0–7199 LPC/10⁶ MNC; Table 2). The LPC values obtained for high-risk patients were significantly higher than those obtained for standard risk patients (P = 0.04).

Overall, 243 (27%) newly diagnosed patients had detectable LPC in their postinduction remission bone marrow samples. By comparison, a significantly larger fraction of relapsed patients (48%) had detectable LPC at the end of reinduction therapy (P = 0.003; Table 2). The LPC content in the relapsed patients (mean ± SE = 202 ± 139 LPC/10⁶ MNC) seemed higher than that of the combined group of newly diagnosed patients (mean ± SE = 22 ± 9 LPC/10⁶ MNC), but this difference reached only borderline significance (P = 0.1). Comparisons of the LPC content of standard risk versus relapsed patients and high-risk versus relapsed patients also reached only borderline significance.

Regardless of risk group, newly diagnosed T-lineage patients were more likely than their B-lineage counterparts to have detectable LPC in their end-of-induction bone marrows (standard risk: B-lineage, 23% versus T-lineage, 64%, P < 0.0001; high risk: B-lineage, 17% versus T-lineage, 74%, P < 0.0001; Table 2). For the combined group of standard and high-risk newly diagnosed patients, the average LPC contents were 9 ± 2/10⁶ MNC for B-lineage ALL patients and 124 ± 71/10⁶ MNC for T-lineage ALL patients (P = 0.05). Comparisons of B-lineage versus T-lineage postinduction bone marrow LPC content for standard risk (P = 0.02) and high-risk patients (P = 0.07) are shown in Table 2. A similar correlation between the postinduction LPC burden and immunophenotype was also observed for patients in first bone marrow relapse. Nineteen of 44 (43%) relapsed B-lineage ALL patients versus 5 of 6 (83%) relapsed T-lineage ALL patients harbored residual LPC in their postinduction bone marrow specimens (P = 0.09; Table 2). However, the average LPC content in relapsed T-lineage patients was not significantly different from that of relapsed B-lineage patients (Table 2).

### In Vivo Chemotherapy Sensitivity of LPC from ALL Patients.
To define the sensitivity of LPC to induction chemotherapy regimens, we compared the preinduction and postinduction bone marrow LPC contents for patients with newly diagnosed and relapsed ALL (Table 3). The log reduction of LPC for newly diagnosed standard risk patients (2.94 ± 0.04) was significantly greater than that of newly diagnosed high-risk patients (2.74 ± 0.06; P = 0.002). The log reductions in LPC were greater for B-lineage patients than for T-lineage patients in both the standard risk subset (B-lineage, 2.97 ± 0.04 versus T-lineage, 2.58 ± 0.14, P = 0.005) and the high-risk subset (B-lineage, 2.89 ± 0.06 versus T-lineage, 2.23 ± 0.14, P < 0.0001), as well as for the combined group newly diagnosed...
The log reductions in LPC were greater for newly diagnosed patients with standard risk (2.94 ± 0.03, P = 0.005) or high-risk ALL (2.74 ± 0.06, P = 0.007) as well as for the combined group of standard and high-risk patients (2.88 ± 0.03, P < 0.001) than for relapsed patients (2.21 ± 0.20, P < 0.001; Table 3). This difference was attributable to differences between newly diagnosed and relapsed patients within the B-lineage subset (P = 0.001), because no significant differences were observed within the T-lineage subset (Table 3). Overall, the order of log reduction of LPC by induction/reinduction chemotherapy was: newly diagnosed standard risk B > newly diagnosed high-risk B-lineage > newly diagnosed standard risk T-lineage > newly diagnosed high-risk T-lineage > relapsed B-lineage > relapsed T-lineage.

**Relationship between Early Marrow Response to Induction Chemotherapy and Log Reduction of LPC in Newly Diagnosed Patients.** In a subset of 486 newly diagnosed standard risk patients with early response data, we examined the relationship between early response as at day 7 of induction therapy and the magnitude of the LPC log reduction at day 28. Among standard risk patients, the average log reduction at the end of induction chemotherapy was greater for the subset of 368 patients (B-lineage, 2.95 ± 0.03 versus T-lineage, 2.35 ± 0.14, P < 0.0001).

### Table 2 Frequency of detection of LPC and LPC content in postinduction bone marrows

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Number of patients with detectable LPC</th>
<th>LPC content of bone marrow</th>
<th>Log kill (mean ± SE)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>A (Mean ± SE)</td>
<td>B (Median (range))</td>
<td>C (Median (range))</td>
</tr>
<tr>
<td><strong>Newly diagnosed standard risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>155/604 (26)</td>
<td>0.1*</td>
<td>2.95 ± 0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>B-lineage</td>
<td>132/568 (23)</td>
<td>&lt;0.0001</td>
<td>2.97 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-lineage</td>
<td>23/36 (64)</td>
<td>NS</td>
<td>2.58 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Newly diagnosed high risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>88/286 (31)</td>
<td>0.02</td>
<td>5.3 ± 1</td>
<td>0.09</td>
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<tr>
<td>B-lineage</td>
<td>38/218 (17)</td>
<td>&lt;0.0001</td>
<td>7.1 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-lineage</td>
<td>50/68 (74)</td>
<td>NS</td>
<td>177 ± 8</td>
<td>NS</td>
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<tr>
<td><strong>Combined newly diagnosed</strong></td>
<td></td>
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<tr>
<td>All patients</td>
<td>243/890 (27)</td>
<td>0.03</td>
<td>22 ± 1</td>
<td>0.10</td>
</tr>
<tr>
<td>B-lineage</td>
<td>170/786 (22)</td>
<td>&lt;0.0001</td>
<td>14 ± 1</td>
<td>&lt;0.001</td>
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<tr>
<td>T-lineage</td>
<td>73/104 (70)</td>
<td>NS</td>
<td>124 ± 7</td>
<td>NS</td>
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<tr>
<td><strong>Relapsed</strong></td>
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<td></td>
<td></td>
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<tr>
<td>All patients</td>
<td>24/50 (48)</td>
<td>0.09</td>
<td>226 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>B-lineage</td>
<td>19/44 (43)</td>
<td>202 ± 139</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>T-lineage</td>
<td>5/6 (83)</td>
<td>25 ± 18</td>
<td>0.06</td>
<td>NS</td>
</tr>
</tbody>
</table>

* P A: standard risk versus high risk; P B: B-lineage versus T-lineage; P C: newly diagnosed versus relapsed.

NS, not significant.

### Table 3 Log reduction of bone marrow LPC content by induction chemotherapy

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Number of patients with detectable LPC</th>
<th>Log kill (mean ± SE)</th>
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<td></td>
</tr>
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<td>2.94 ± 0.04</td>
<td>0.002</td>
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<tr>
<td>B-lineage</td>
<td>132 (23)</td>
<td>2.97 ± 0.04</td>
<td>NS*</td>
</tr>
<tr>
<td>T-lineage</td>
<td>23 (64)</td>
<td>2.58 ± 0.14</td>
<td>0.04</td>
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<tr>
<td><strong>Newly diagnosed high risk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>88 (31)</td>
<td>2.74 ± 0.06</td>
<td>0.007</td>
</tr>
<tr>
<td>B-lineage</td>
<td>38 (17)</td>
<td>2.89 ± 0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T-lineage</td>
<td>50 (74)</td>
<td>2.23 ± 0.14</td>
<td>NS</td>
</tr>
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<td><strong>Combined newly diagnosed</strong></td>
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<td>0.001</td>
</tr>
<tr>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>T-lineage</td>
<td>73 (70)</td>
<td>2.35 ± 0.14</td>
<td>NS</td>
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<tr>
<td><strong>Relapsed</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>24 (48)</td>
<td>2.21 ± 0.20</td>
<td>0.001</td>
</tr>
<tr>
<td>B-lineage</td>
<td>19 (43)</td>
<td>2.23 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>T-lineage</td>
<td>5 (83)</td>
<td>2.12 ± 0.37</td>
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</table>

* P A: standard risk versus high risk; P B: B-lineage versus T-lineage; P C: newly diagnosed versus relapsed.

NS, not significant.
RER patients (mean ± SE, 3.02 ± 0.05 logs; median, 3.29 logs) than for the subset of 118 SER patients (mean ± SE, 2.78 ± 0.09 logs; median, 2.99 logs; \( P = 0.008 \)). In contrast, among the 183 higher-risk patients, the LPC log reduction values were not significantly different for the 139 RER patients (mean ± SE, 2.62 ± 0.12 logs; median, 2.92 logs) and the 44 SER patients (mean ± SE, 2.95 ± 0.12 logs; median, 2.96 logs; Table 4).

**Relationship between End-Of-Induction Leukemia Burden and EFS of Newly Diagnosed Patients.** As shown in Fig. 1, patients whose end-of-induction remission bone marrow specimens had no detectable LPC had an excellent early EFS outcome. Within the standard risk subset \( (n = 599) \), patients with zero LPC in their end-of-induction bone marrow specimens \( \text{i.e.}, \) LPC\(^-\) patients, \( n = 445 \) had a 2.6-fold lower incidence of events than patients whose end-of-induction bone marrow specimens had detectable LPC \( \text{i.e.}, \) LPC\(^+\) patients, \( n = 154; 11 \text{ of } 445, 2.5\% \text{ versus } 10 \text{ of } 154, 6.5\%; \) \( P = 0.025 \); Fig. 1, A and C). The probability of EFS at 24 months from study entry was 96.9 ± 1.0% for the LPC\(^-\) group and 92.7 ± 2.3% for the LPC\(^+\) group \( (P = 0.045) \). Similarly, within the high-risk subset \( (n = 276) \), patients with zero LPC in their end-of-induction bone marrow specimens \( \text{i.e.}, \) LPC\(^-\) patients, \( n = 190 \) had a 2.4-fold lower incidence of events than patients whose end-of-induction bone marrow specimens had detectable LPC \( \text{i.e.}, \) LPC\(^+\) patients, \( n = 86; 10 \text{ of } 190, 5.3\% \text{ versus } 11 \text{ of } 86, 12.8\%; \) \( P = 0.046 \); Fig. 1, B and C). The probability of EFS at 24 months from study entry was 92.6 ± 2.4% for the LPC\(^-\) group and 78.9 ± 6.3% for the LPC\(^+\) group \( (P = 0.022) \). At 6 months, 12 months, as well as 24 months, the ranking order for better EFS was: standard risk, LPC\(^-\) > high risk, LPC\(^+\) > standard risk, LPC\(^+\) > high risk, and LPC\(^+\) \( (\text{Fig. } 1C) \). Thus, LPC\(^+\) standard risk patients had a worse early EFS outcome than LPC\(^-\) high-risk patients, and LPC\(^-\) high-risk patients had a better early EFS outcome than LPC\(^+\) standard risk patients.

**DISCUSSION**

In recent years, several laboratories have developed sensitive detection methods to discern small numbers of residual leukemic cells in remission bone marrow samples from ALL patients \( (18–38) \). Such methods provide a way to evaluate the quality of remission in children with ALL who are treated on contemporary chemotherapy programs. Monitoring of MRD during complete morphological remission may help us predict which patients are likely to relapse, based on certain levels of MRD. Identification of such patients might allow initiation of alternative treatment modalities designed to improve their outcome. Such measurements of MRD may also provide a valuable surrogate marker for rapid assessment of novel therapeutic interventions in Phase II and III trials. Numerous methods, including detection of IgM and T-cell receptor gene rearrangements by PCR, identification of leukemic-specific surface antigen profiles by multiparameter flow cytometry, and detection of specific chromosomal abnormalities by fluorescent in situ hybridization are being tested for their abilities to predict relapse based on the quantification of residual leukemia during complete remission \( (18–38) \). Similar to the results obtained in our study with FACS/LPC, these techniques have detected residual leukemic blasts in 25–40% of patients at the end of induction \( (21, 24) \). Furthermore, several investigators have reported positive correlations between the amount of MRD, either at the end of induction therapy or during later stages of therapy, and risk of relapse \( (21, 22, 33, 34) \).

We have focused our efforts on the use of a quantitative test that combines multiparameter flow cytometry and cell sorting with LPC assays. The quantitative MRD detection system using LPC assays does not require the presence of clonal chromosomal abnormalities or the availability of clonospecific probes. Therefore, it provides an opportunity to routinely analyze remission ALL bone marrow specimens for MRD by identifying residual clonogenic blasts capable of *in vitro* proliferation and blast colony formation. In a preliminary study of 83 high-risk ALL patients undergoing autologous BMT in first remission, multivariate analysis of all competing covariates established the residual LPC burden in pretransplant remission bone marrow as a reliable predictor of relapse after autologous BMT \( (41) \). These findings indicated that the determination of the LPC count in remission bone marrows could be clinically useful as a measure of the quality of remission. In two more recent studies, we used this MRD detection method to quantify occult disease in the bone marrows of pediatric patients with ALL who had an isolated extramedullary first relapse. We found that more than half of the patients with B-lineage ALL tested lacked detectable LPC in the bone marrow at the time of extramedullary relapse and that, among those with detectable LPC, reinduction chemotherapy was capable of reducing the LPC content in the bone marrow to below detection levels \( (42) \). By comparison, more than half of the patients with extramedullary relapse and T-lineage ALL had substantial occult involvement in the bone marrow that was refractory to reinduction chemotherapy \( (43) \). These data suggested that T-lineage ALL patients who experience an isolated extramedullary first relapse might be at higher risk for subsequent bone marrow relapse.

In the present study, we evaluated the quality of first and second remission in a large series of newly diagnosed and relapsed pediatric ALL patients. We also compared the *in vivo* chemotherapy sensitivity of LPCs, as measured by log reduction of pretreatment bone marrow LPC content after induction chemotherapy, in relationship to NCI risk classification, immunophenotype, day 7 bone marrow status, and disease status \( \text{i.e.}, \) initial diagnosis versus first relapse. Overall, the reinduction chemotherapy of the CCG-1941 salvage protocol was significantly less effective against LPC from relapsed patients than were the frontline three-drug or four-drug induction chemotherapy regi-

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**Table 4** Log reduction in bone marrow LPC according to early treatment response

| Risk group | \( n \) | Mean ± SE | Median (range) | \( P \)
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<tr>
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<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RER</td>
<td>368</td>
<td>3.02 ± 0.05</td>
<td>3.29 (0–5.11)</td>
<td>0.008</td>
</tr>
<tr>
<td>SER</td>
<td>118</td>
<td>2.78 ± 0.09</td>
<td>2.99 (0–4.52)</td>
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<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RER</td>
<td>139</td>
<td>2.70 ± 0.10</td>
<td>3.07 (0–4.13)</td>
<td>NS</td>
</tr>
<tr>
<td>SER</td>
<td>44</td>
<td>2.95 ± 0.12</td>
<td>3.01 (0.06–4.03)</td>
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\( a \) *P* comparing RER and SER.

\( b \) NS, not significant.
mens against LPC from standard risk and high-risk newly diagnosed patients. Consequently, the LPC burden of postinduction bone marrows from patients in first bone marrow relapse was \( \sim 10 \)-fold greater \( (\sim 200/10^6 \text{ MNC}) \) than the LPC burden of postinduction bone marrows from newly diagnosed patients \( (\sim 20/10^6 \text{ MNC}) \). Furthermore, significantly fewer newly diagnosed patients had detectable LPC in the postinduction bone marrow samples than did relapsed patients. These results show that the quality of first bone marrow remission after the frontline induction chemotherapy is better than the quality of the second bone marrow remission after reinduction chemotherapy. The incorporation of new agents with novel mechanisms of action may improve the efficacy of reinduction chemotherapy regimens for patients with early bone marrow relapse. Alternatively, postinduction intensification regimens using new agents may help eradicate the residual leukemia burden refractory to reinduction chemotherapy.

Among newly diagnosed patients, induction chemotherapy resulted in a significantly greater log reduction in LPC burden for the standard risk group than for the higher-risk group. The average LPC burden of the postinduction chemotherapy bone marrows from high-risk ALL patients was \( \sim 7 \)-fold greater than the average LPC burden of the postinduction bone marrows from standard risk ALL patients. These results are consistent with the notion that patients with higher-risk ALL require more intensive postinduction chemotherapy than do standard risk patients to eradicate the residual leukemic cells that escape the induction chemotherapy.

Notably, a greater fraction of newly diagnosed T-lineage patients than newly diagnosed B-lineage patients had detectable LPC in their postinduction bone marrows, regardless of NCI risk classification. LPC of patients with newly diagnosed T-lineage ALL seemed to be no more sensitive to frontline induction chemotherapy than were LPC of relapsed T-lineage patients to intensive reinduction chemotherapy. Nonetheless, 75% of patients with T-cell ALL are long-term survivors when treated on current CCG protocols. These findings suggest that subsequent consolidation, delayed intensification, and maintenance phases of CCG treatments protocols are able to eliminate residual leukemic blasts that escaped induction therapy.

We also examined the relationship between day 7 early marrow response to induction chemotherapy and the magnitude of the LPC load reduction. LPC of patients with standard risk ALL who have a slow early marrow response seem to be, on average, more resistant to the three-drug induction chemotherapy than patients who have a rapid early marrow response. In contrast, very similar LPC log reduction values were obtained for high-risk patients receiving more intensive four-drug induction chemotherapy, regardless of their early marrow response. However, within both the SER and RER subsets, there was a five-log variation in chemosensitivity of LPC. The clinical significance of this marked interpatient variation remains to be determined, but patients with more resistant LPC may be at higher risk for subsequent relapse.

In summary, our findings revealed a marked heterogeneity relative to the LPC content of the postinduction bone marrows.
as well as chemosensitivity of LPC. NCI risk classification, immunophenotype, disease status, as well as early marrow response showed intriguing correlations with the LPC assay data. Overall, the order of chemosensitivity of LPC was: newly diagnosed standard risk B-lineage ALL > newly diagnosed high-risk B-lineage ALL > newly diagnosed standard risk T-lineage ALL > newly diagnosed high-risk T-lineage ALL > relapsed B-lineage ALL > relapsed T-lineage ALL. Notably, LPC+ patients whose end-of-induction remission bone marrow specimens had zero LPC had an excellent early EFS outcome. Within the standard and high-risk subsets, LPC+ patients had a 2.6-fold lower and 2.4-fold lower incidence of events, respectively, than LPC- patients. At 6 months, 12 months, as well as 24 months, the ranking order for better EFS was: standard risk, LPC- > high risk, LPC- > standard risk, LPC+ > high risk, LPC+. Thus, LPC+ standard risk patients had a worse early EFS outcome than LPC+ high-risk patients, and LPC+ high-risk patients had a better early EFS outcome than LPC- standard risk patients. Whereas the 24 months follow-up is too short to arrive at accurate conclusions regarding the probability of long-term EFS, these results are in agreement with the previously reported predictive value of the LPC-based MRD measurements (41).

REFERENCES

Residual Bone Marrow Leukemic Progenitor Cell Burden after Induction Chemotherapy in Pediatric Patients with Acute Lymphoblastic Leukemia

Fatih M. Uckun, Linda Stork, Nita Seibel, et al.