Clinical Significance of Novel Subtypes of Acute Lymphoblastic Leukemia in the Context of Minimal Residual Disease–Directed Therapy

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ABSTRACT

We evaluated clinical significance of recently identified subtypes of acute lymphoblastic leukemia (ALL) in 598 children treated with minimal residual disease (MRD)-directed therapy. Among the 16 B-cell ALL (B-ALL) and 8 T-cell ALL subtypes identified by next-generation sequencing, ETV6–RUNX1, high-hyperdiploid, and DUX4-rearranged B-ALL had the best 5-year event-free survival rates (95.0%–98.4%); TCF3–PBX1, PAX5-altered (PAX5alt), T-cell, early T-cell precursor (ETP), intrachromosomal amplification of chromosome 21 (iAMP21), and hypodiploid ALL intermediate rates (80.0%–88.2%); and BCR–ABL1, BCR–ABL1-like, ETV6–RUNX1-like, and KMT2A-rearranged ALL the worst rates (64.1%–76.2%). All but 3 of the 142 patients with day 8 blood MRD <0.01% remained in remission. Among new subtypes, intensified therapy based on day 15 MRD ≥1% improved outcome of DUX4-rearranged, BCR–ABL1-like, and ZNF384-rearranged ALL, and achievement of day 42 MRD <0.01% did not preclude relapse of PAX5alt, MEF2D-rearranged, and ETV6–RUNX1-like ALL. Thus, new subtypes including DUX4-rearranged, PAX5alt, BCR–ABL1-like, ETV6–RUNX1-like, MEF2D-rearranged, and ZNF384-rearranged ALL have important prognostic and therapeutic implications.

SIGNIFICANCE: Genomic analyses and MRD should be used together for risk-directed treatment of childhood ALL. Six recently described subtypes—DUX4-rearranged, PAX5alt, BCR–ABL1-like, ETV6–RUNX1-like, MEF2D-rearranged, and ZNF384-rearranged ALL—had prognostic and therapeutic significance with contemporary risk-directed treatment.

See related commentary by Segers and Cools, p. 294.

See related video from the AACR Annual Meeting 2021: https://vimeo.com/558556916

Note: Supplementary data for this article are available at Blood Cancer Discovery Online (https://bloodcancerdiscov.aacrjournals.org/).

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Blood Cancer Discov 2021;2:326–37
doi: 10.1158/2643-3230.BCD-20-0229
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INTRODUCTION

Childhood acute lymphoblastic leukemia (ALL) is one of the most curable cancers, with 5-year event-free survival rates exceeding 80% in many developed countries (1). Precise assessment of the early treatment response based on measurement of minimal residual disease (MRD) for risk-directed therapy has contributed significantly to this success (2). In randomized trials, MRD-directed treatment improved event-free survival by augmenting postremission therapy in patients with persistent MRD at the end of remission induction and by reducing treatment intensity in low-risk patients with rapid early clearance of MRD (3, 4). Accurate identification of patients with highly curable leukemia provides unique opportunities for further reduction in treatment intensity, thus decreasing the likelihood of short-term morbidity and mortality as well as long-term sequelae (4, 5). The relative risk of relapse among patients with early MRD clearance appears to differ among leukemia subtypes (6, 7). In the AIEOP-BFM 2000 study, for example, standard-risk patients who were MRD negative on days 33 and 78 of induction were randomized to receive reduced-intensity treatment in the delayed intensification phase, but this modification was successful only for patients with ETV6–RUNX1 and those who were 1 to 6 years old (8).

Recent integrated genomic analyses, especially transcriptome sequencing, have identified several new subtypes of ALL, including BCR–ABL1-like, DUX4-rearranged, ETV6–RUNX1-like, MEF2D-rearranged, PAX5-altered (PAX5alt), and ZNF384-rearranged ALL (9–13). The clinical significance of some of these novel subtypes, however, is uncertain as they were identified retrospectively among selected patient cohorts that had received a variety of treatment regimens, the intensity of which was not consistently based on MRD levels (9–13). In this study, we evaluated the prognostic and therapeutic implications of all leukemia subtypes identifiable by genetic and transcriptomic analyses including nine B-cell (B-ALL) and eight T-cell ALL (T-ALL) subtypes not identifiable by conventional cytogenetic analysis among consecutive patients who had comprehensive genomic analyses and were treated on a contemporary risk-directed protocol based on well-recognized genetic abnormalities and MRD assessment at three time points during remission induction (14).

RESULTS

Risk Assignment and Genomic Classification

Of the 598 evaluable patients enrolled in St. Jude Total Therapy Study 16, 260 were classified to have low-risk, 280
standard-risk, and 58 high-risk ALL based on presenting clinical and biological features and MRD levels on days 15 and 42 of remission induction (Supplementary Fig. S1; Table 1). For B-ALL, genomic analyses identified 16 leukemia subtypes defined by recurring genetic alterations and distinct gene-expression profiles, 9 of which could not be reliably identified with conventional methods and required transcriptomic sequencing analysis for accurate identification: BCL2/MYC, BCR–ABL1-like, DUX4-rearranged, ETV6–RUNX1-like, MEF2D-rearranged, NUTM1-rearranged, PAX5 P80R, and ZNF384-rearranged (Table 1; Supplementary Figs. S2–S4). The demographic characteristics, sequential MRD levels, treatment risk group, and clinical outcomes for patients with each leukemia subtype are provided in Supplementary Table S1. Most patients with ETV6–RUNX1 or high-hyperdiploid ALL having low levels of MRD measured at three time points during remission induction (Fig. 1) were treated in the low-risk group, all patients with BCR–ABL1 or early T-cell precursor (ETP) ALL in the high-risk group, and most patients with other subtypes in the standard-risk group (Table 1). “B other” comprised B-ALL cases that could not be classified by cytogenetic, genetic, or transcriptomic analyses.

### Treatment Outcome by Leukemia Subtypes

The entire cohort of 598 patients had a 5-year event-free survival of 88.8% [95% confidence interval (CI), 85.9–91.7], overall survival of 94.0% (91.8–96.2), and cumulative risk of any relapse of 7.4% (5.3–9.6). Based on their highest event-free survival rates (Table 1; Fig. 2), ETV6–RUNX1, high-hyperdiploid, and DUX4-rearranged B-ALL were categorized as favorable
subtypes (Supplementary Fig. S5); these three subtypes also have the highest overall survival rates (Supplementary Fig. S6) and the lowest relapse rates (Table 1). Notably, only 13.3% of patients with \textit{ETV6–RUNX1} abnormality and 33.1% of those with high hyperdiploidy but 60% of patients with \textit{DUX4} rearrangement received standard-risk treatment, suggesting that MRD assessment improved the outcome of these patients by avoiding overtreatment or undertreatment.

\textit{BCR–ABL1}, \textit{BCR–ABL1-like}, \textit{ETV6–RUNX1-like}, \textit{KMT2A}-rearranged, and \textit{MEF2D}-rearranged ALL had high levels of MRD (Fig. 1) and were categorized to be unfavorable subtypes because of their worst event-free survival rates (Table 1; Fig. 2). The remaining subtypes including \textit{TCF3–PBX1}, \textit{PAX5alt}, T-cell, ETP, intrachromosomal amplification of chromosome 21 (iAMP21), hypodiploid, \textit{ZNF384}-rearranged, \textit{NUTM1}-rearranged, and \textit{PAX5} P80R ALL were considered to have intermediate risk (Supplementary Fig. S5). The \textit{BCL2}/\textit{MYC} group was composed of only one case and therefore not included in downstream analyses.

**Impact of Peripheral Blood MRD Levels on Day 8**

Day 8 MRD levels were <0.01% in 142 (24.8%) of the 572 patients with available data (Supplementary Table S2). Notably, all but three of these patients (two with \textit{KMT2A}-rearranged and one with \textit{TCF3–PBX1} ALL) remained in continuous complete remission. The proportion of patients with a day 8 MRD <0.01% ranged widely across leukemia subtypes, from 0% to 51.2% (Supplementary Table S2). The day 8 MRD finding did not correlate significantly with outcome within individual leukemia subtypes, except for high-hyperdiploid ALL. Among leukemia subtypes associated with the lowest risk of relapse, a day 8 MRD <0.01% was found in 51.2% of patients with \textit{ETV6–RUNX1} and 21.1% of those with high-hyperdiploid ALL, but in none of those with \textit{DUX4}-rearranged ALL.

**Impact of Bone Marrow MRD Levels on Day 15**

MRD levels on day 15 were <0.01% in 187 (31.7%), 0.01% to <1% in 226 (38.3%), and ≥1% in 177 (30.0%) of the 590 patients tested (Fig. 3A; Table 2). Overall, patients with a day 15 MRD ≥1% had significantly worse 5-year event-free survival and higher cumulative risk of relapse than those with lower or undetectable MRD levels (P < 0.001). However, high MRD on day 15 conferred a significantly poorer 5-year event-free survival only in cases with high-hyperdiploid ALL (P = 0.05) and \textit{B} other ALL (P < 0.001), which consisted of heterogeneous diseases (Table 2). In patients with other leukemia subtypes, day 15 MRD ≥1% lacked prognostic impact, which could be due to treatment intensification triggered by this MRD finding and small number of patients in some subtypes. With standard-risk or high-risk treatment, relapse did not occur in any of the 36 patients with day 15 MRD ≥1% and \textit{ETV6–RUNX1}, \textit{DUX4}-rearranged, iAMP21, hypodiploid, \textit{BCR–ABL1-like}, or \textit{ZNF384}-rearranged ALL (Supplementary Table S3), again suggesting that subsequent intensification of treatment improved their outcome.

**Impact of Bone Marrow MRD Levels on Day 42**

Day 42 MRD levels were 0.01% to <1% in 60 (10.2%) of the patients and ≥1% in only 15 (2.6%; Fig. 3B; Table 3). Patients who attained a day 42 MRD <0.01% had a significantly better outcome than those with levels of 0.01% to <1%, who in turn fared better than patients with MRD ≥1% (P < 0.001). Among the 279 patients with favorable genotypes (\textit{ETV6–RUNX1}, high hyperdiploidy, or \textit{DUX4} rearrangement) who attained day 42 MRD <0.01%, 2 relapsed with a 5-year cumulative risk of relapse of 1.3% (0-2.8; Table 3). By contrast, of the 184 patients with intermediate-risk or unfavorable subtypes and day 42 MRD <0.01%, 20 including 4 with \textit{PAX5alt} ALL and
determination during remission induction have important prognostic and therapeutic implications. Our data indicate that patients with certain genetic ALL subtypes are almost always curable with conventional chemotherapy guided by early MRD assessment. In our study, 5-year overall survival for patients with ETV6–RUNX1-positive or high-hyperdiploid ALL exceeded 99% (99.2% (95% CI, 97.4–100) and 99.4% (97.8–100), respectively). In the study by Lilljebjörn and colleagues (10), relapse was observed in 4 of 28 DUX4-rearranged patients, whereas in our study, despite elevated early MRD in 12 (60%) cases, the only adverse event in the DUX4-rearranged cohort was fatal sepsis, resulting in a 5-year event-free survival of 95.0% (84.2–100). MRD of less than 0.01% in peripheral blood on day 8 of induction treatment by itself identified a subgroup with an excellent outcome. Among the 142 patients with this early finding, only 3 (2 with KMT2A-rearranged and 1 with TCF3–PBX1 ALL) relapsed. None of the 95 patients with either ETV6–RUNX1 or high-hyperdiploid ALL who had a day 8 MRD <0.01% in blood and received low-risk therapy relapsed, suggesting that patients with these features should be considered for further treatment reduction in future trials. Our data, however, should not be interpreted to support treatment reduction in patients with other ALL subtypes even if they achieve a day 8 MRD <0.01%, as 39 of the 47 patients in this subgroup received standard- or high-risk therapy in our study.

The prognostic significance of MRD levels in peripheral blood on day 8 of induction has also been evaluated in other studies. Among patients who received Berlin–Frankfurt–Münster (BFM) backbone treatment regimens, the day 8 MRD result in blood after 1 week of pre-phase prednisone therapy and intrathecal methotrexate had little prognostic impact.
(15–17). Among B-ALL patients treated in the COG P9900 protocols, however, a day 8 MRD ≤0.01% in blood after three- or four-drug induction plus intrathecal therapy was associated with a better event-free survival, while increasing levels of MRD at that time point were associated with a progressively worse outcome (18, 19). Because flow-cytometric measurements of MRD can be simplified when applied at early time points during remission induction therapy, particularly in peripheral blood (20), and a reduction in the intensity of remission induction therapy in low-risk patients was highly successful in a recent study (21), the day 8 MRD finding in blood could be used together with an uncomplicated genetic analysis (22) to identify low-risk patients for treatment reduction. This strategy would be especially effective in low-
middle-income countries to decrease the rates of induction death and treatment abandonment.

In this study, MRD measured in bone marrow on day 15 of remission induction was useful to identify patients with a poor early response who may have otherwise been regarded as having low-risk ALL for treatment intensification. Thus, none of the 7 ETV6–RUNX1 and 10 DUX4-rearranged patients, and only 2 of the 37 high-hyperdiploid patients who received standard-risk treatment because of MRD ≥1% on day 15, subsequently relapsed. Treatment intensification based on MRD ≥1% on day 15 also appeared to be beneficial for patients with intermediate-risk or unfavorable genetic subtypes. With standard- or high-risk treatment, relapse did not occur in any patient with iAMP21, ZNF384-rearranged, hypodiploid <44, or BCR–ABL1-like ALL and a day 15 MRD <0.01%. Notably, achievement of undetectable (<0.01%) MRD on day 42 did not preclude subsequent relapse in patients with intermediate-risk or unfavorable subtypes, including TCF3–PBX1, PAX5alt, T-cell, iAMP21, BCR–ABL1, BCR–ABL1-like, ETV6–RUNX1-like, KMT2A-rearranged, or MEF2D-rearranged ALL. It is possible that more sensitive MRD assays, such as deep sequencing analysis, could identify patients at a higher risk of relapse among those with a negative MRD finding according to the most widely used cutoff of 0.01% (23, 24).

<p>| Table 2. Treatment outcome based on leukemia cell subtype and MRD in bone marrow at day 15 of induction |</p>
<table>
<thead>
<tr>
<th>Number of patients (%)</th>
<th>5-year EFS, % (95% CI)</th>
<th>5-year CRR, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRD &lt;0.01%</td>
<td>MRD 0.01% to &lt;1%</td>
</tr>
<tr>
<td>ETV6–RUNX1</td>
<td>68 (53.5)</td>
<td>52 (40.9)</td>
</tr>
<tr>
<td>High-hyperdiploid</td>
<td>47 (30.9)</td>
<td>68 (44.7)</td>
</tr>
<tr>
<td>DUX4-rearranged</td>
<td>0 (0.00)</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>TCF3–PBX1</td>
<td>4 (25.0)</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>PAX5alt</td>
<td>6 (26.1)</td>
<td>8 (34.8)</td>
</tr>
<tr>
<td>T-cell</td>
<td>23 (24.7)</td>
<td>29 (31.2)</td>
</tr>
<tr>
<td>ETP</td>
<td>0 (0.00)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>iAMP21</td>
<td>1 (20.0)</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>0 (0.00)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>BCR–ABL1</td>
<td>1 (7.69)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>BCR–ABL1-like</td>
<td>3 (20.0)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>ETV6–RUNX1-like</td>
<td>2 (22.2)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>KMT2A-rearranged</td>
<td>6 (21.4)</td>
<td>11 (39.3)</td>
</tr>
<tr>
<td>MEF2D-rearranged</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>ZNF384-rearranged</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>NUTM1-rearranged</td>
<td>3 (100)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>PAX5P8OR</td>
<td>1 (50.0)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>B other</td>
<td>21 (35.6)</td>
<td>22 (37.3)</td>
</tr>
<tr>
<td>Total</td>
<td>187 (31.7)</td>
<td>226 (38.3)</td>
</tr>
</tbody>
</table>

Abbreviations: CRR, cumulative risk of any relapse; EFS, event-free survival.
Leukemia Subtypes and MRD in Childhood ALL

Table 3. Treatment outcome based on leukemia cell subtype and MRD in bone marrow at day 42 (end of induction)

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>5-year EFS, % (95% CI)</th>
<th>5-year CRR, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRD</strong></td>
<td><strong>0.01% to &lt;1%</strong></td>
<td><strong>≥1%</strong></td>
</tr>
<tr>
<td>ETV6–RUNXI</td>
<td>115 (91.3)</td>
<td>11 (8.73)</td>
</tr>
<tr>
<td>High-hypodiploid</td>
<td>145 (94.8)</td>
<td>8 (5.23)</td>
</tr>
<tr>
<td>DUX4-rearranged</td>
<td>19 (95.0)</td>
<td>1 (5.00)</td>
</tr>
<tr>
<td>7CF3–PBX1a</td>
<td>14 (87.5)</td>
<td>1 (6.25)</td>
</tr>
<tr>
<td>PAX5altb</td>
<td>16 (66.7)</td>
<td>8 (33.3)</td>
</tr>
<tr>
<td>T-cell</td>
<td>76 (82.6)</td>
<td>9 (7.98)</td>
</tr>
<tr>
<td>ETP</td>
<td>6 (66.7)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>IAMP21</td>
<td>5 (100)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>BCR–ABL1</td>
<td>11 (84.6)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>BCR–ABL1-likec</td>
<td>9 (60.0)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>ETV6–RUNXI-likec</td>
<td>7 (77.8)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>KMT2A-rearranged</td>
<td>20 (80.0)</td>
<td>5 (20.0)</td>
</tr>
<tr>
<td>MEF2D-rearrangedd</td>
<td>3 (100)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>ZNF384-rearranged</td>
<td>7 (100)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>NUTM2-rearranged</td>
<td>3 (100)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>PAX5 P80R</td>
<td>2 (100)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Other</td>
<td>49 (83.1)</td>
<td>8 (13.6)</td>
</tr>
<tr>
<td>Total</td>
<td>512 (87.2)</td>
<td>60 (10.2)</td>
</tr>
</tbody>
</table>

Abbreviations: CRR, cumulative risk of any relapse; EFS, event-free survival.

aAmong patients with TCF3–PBX1 ALL, one with day 42 MRD <0.01% relapsed at 5.7 years, and two with positive MRD died of transplant-related toxicities at 0.6 and 2.4 years, respectively.

bOf the 16 PAX5Salt patients with day 42 MRD <0.01%, 4 relapsed [2 hematologic and 2 central nervous system (CNS) relapses].

cOf the nine BCR–ABL1-like patients with day 42 MRD <0.01%, one developed CNS relapse.

dOf the seven ETV6–RUNXI-like patients with day 42 MRD <0.01%, one had hematologic relapse, and another developed myelodysplastic syndrome.

so, such patients might be considered as candidates for novel targeted therapies (25, 26).

Our study suggests that several newly identified genotypes might be prognostically relevant in the context of contemporary risk-directed treatment. Conceivably, DUX4-rearranged ALL (9, 10) could join ETV6–RUNXI and high-hypodiploid ALL as one of the most favorable subtypes. Although none of our 20 patients with this feature relapsed, it should be noted that 12 of them received standard-risk therapy because of day 15 MRD >1%. Patients with PAX5Salt ALL, commonly classified...
as having high-risk ALL by NCI criteria because of presenting age above 10 years or leukocyte count above 10 × 10^9/μL, had a 5-year event-free survival of 71.5% ± 7.0% when treated in the Children’s Oncology Group AALL0232 protocol for high-risk ALL (13). In our study, 2 of the 24 patients with PAX5alt ALL developed hematologic relapse and two central nervous system (CNS) relapse, with a 5-year event-free survival of 82.7% (65.3–100). Although they had a day 42 MRD <0.01%, all four relapsed patients were treated with standard-risk therapy because of unfavorable presenting clinical features (age >10 years in two patients, leukocyte count 225 × 10^9/μL in one) or a poor early treatment response (day 15 MRD >1% in one). Hence, we consider this subtype to have an intermediate risk of relapse.

In the first report of ETV6–RUNXI-like ALL, 2 of the 10 patients relapsed (10). Among our nine patients with this genotype, seven were treated with standard-risk therapy, two of whom relapsed (one with a day 42 MRD <0.01% and two were treated with low-risk therapy, one of whom developed myelodysplastic syndrome. Likewise, both of our relapsed MEF2D-rearranged and IAMP21 patients had a day 42 MRD <0.01%; both genotypes have been associated with an increased risk of relapse (11, 27, 28). Notably, our relapsed patient with MEF2D-rearranged ALL was also treated with standard-risk therapy. Thus, an MRD <0.01% at the end of induction does not ensure high curability of patients with several recently identified genetic subtypes, even in the context of contemporary risk-directed therapy. Additional studies of a larger number of patients are needed to confirm our findings and to determine whether patients with these subtypes can benefit from additional molecularly targeted therapy, immunotherapy, or both.

Transcriptome sequencing analyses in this study identified patients with three other uncommon subtypes: ZNF384-rearranged, NUTM1-rearranged, and PAX5 P80R ALL. Our previous study suggested that, despite expression of B- and myeloid lineage markers, ZNF384-rearranged cases should be treated as ALL based on the similarity of their genomic landscape to that of B-ALL (29). In two small series, these patients had 5-year event-free survival rates of 50% to 83% (11, 30). All seven cases in this study remained in remission for 6.8 to 11.5 years, but they were all treated with standard-risk therapy owing to a day 15 MRD >1%. NUTM1-rearranged ALL is a rare B-ALL subtype, and while all seven patients reported in one series were in continuous remission, four received treatment for intermediate- to high-risk ALL (31). In this study, all three NUTM1-rearranged patients were in remission after standard-risk treatment. PAX5 P80R is a recently identified B-ALL subtype with a 5-year event-free survival of 75.0% ± 7.0% in the eight patients treated in the Children’s Oncology Group AALL0232 study, and 50.0% ± 17.7% in the six patients treated in the St. Jude Total Therapy studies (13). For these reasons, we believe that all three subtypes have an intermediate-risk prognosis—an impression requiring confirmation.

Several of the novel subtypes have immunophenotypic features suggestive of the diagnosis: CD2 and CD371 positivity in DUX4-rearranged ALL (32), CD10 negativity and CD28 positivity in MEF2D-rearranged ALL (27), and aberrant myeloid antigen expression in ZNF384-rearranged ALL (29). With the exception of CD371, none of the other features are specific for the associated subtypes, and some level of genomic analysis is required for accurate diagnosis. Moreover, ZNF384 rearrangement defines a broader entity comprising B-progenitor ALL (such cases may have aberrant myeloid marker expression, but not myeloperoxidase) and B/myeloid mixed phenotype acute leukemia (myeloperoxidase positive; ref. 29).

T-ALL can be divided into subtypes by gene-expression profiling or by mutated functional pathway; some cases had rare ABL class fusions (e.g., NUP214–ABL1) that may respond to a tyrosine kinase inhibitor (26). Unlike B-ALL, T-ALL lacks consensus genetic classification with prognostic or therapeutic significance. Inconsistently, NOTCH1 and FBXW7 mutations were associated with favorable prognosis, whereas Ras mutation, PTEN mutation, and lack of biallelic TRG rearrangement (as a surrogate for immature, early T-cell precursor ALL) were associated with unfavorable prognosis (26). An important future study will be comprehensive consideration of gene expression, sequence, and structural cohorts in adequately powered studies of uniformly treated T-ALL to examine the interaction of subtype and secondary mutations and outcome in T-ALL. In the Children’s Oncology Group AALL0434 study, based on the expression of various transcription factors and event-free survival, T-ALL cases were grouped into low-risk (NKX2, HOXA, TAL2, and TLX1), intermediate-risk (LMO2-LYL1, TLX3, and TAL1), and high-risk (LMO1/2, ABL1, and KMT2A-rearranged) categories (26, 33). In this study, we could confirm the poor prognosis of patients in the LMO1/2 subgroup, but our patients in the HOXA group (with or without KMT2A rearrangement) had a high cumulative risk of relapse resulting in low event-free survival. Additional studies are needed to determine the prognosis of patients with HOXA expression.

Together, our results suggest that both systematic genomic analyses and MRD measurements are required to accurately stratify children with ALL into risk groups and tailor their therapy accordingly. We have adopted this approach in our current Total Therapy Study 17. Our data showing poor prognosis of several newly identified subtypes of B-ALL despite very intensive therapy emphasize the need to expand the application of immunotherapy and novel mutation-, fusion gene-, or pathway-directed treatments to leukemia variants resistant to conventional treatment. Because of the small number of patients studied, additional studies are needed to evaluate the prognostic and therapeutic relevance of ETV6–RUNXI-like, ZNF384-rearranged, and MEF2D-rearranged B-ALL, and T-ALL with HOXA expression.

**METHODS**

**Patients and Risk Classification**

From October 29, 2007, to March 26, 2017, 598 eligible patients ages between 0.12 and 18.9 years (median, 6.04) with newly diagnosed ALL were enrolled in Total Therapy Study 16 (ClinicalTrials.gov Identifier NCT00549848) at St. Jude Children’s Research Hospital (14). The trial protocol was approved by the institutional review board and is available in the Supplementary Information. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from the parents or guardians and assent from the patients, as appropriate.

The diagnosis of ALL was based on the immunophenotypic and genetic characteristics of the leukemic cells (14). Genomic classification was based on cytogenetics: FISH for ETV6–RUNXI, TCF3–PBX1,
Leukemia Subtypes and MRD in Childhood ALL

BCR–ABL1, and KMT2A rearrangement and transcriptome sequencing [RNA sequencing (RNA-seq)] where available (n = 502; ref. 13). Details for genomic classification are provided in Supplementary Figs. S2–S4. MRD levels were determined by flow cytometry (14, 34) in blood samples on day 8 and in bone marrow samples on days 15 and 42 (the end of remission induction); a negative MRD was defined as a level <0.01%.

Patients with B-ALL between 1 and 10 years and with a blood leukocyte count at presentation <50 × 10⁹/μL, DNA index ≥1.16 (high hyperdiploidy), or ETV6–RUNX1 fusion were provisionally classified as having low-risk ALL. Those with MRD ≥1% on day 15 of induction or <1% on day 42 were classified as having standard (intermediate)-risk ALL. Patients with the BCR–ABL1 or ETP ALL, infants with KMT2A rearrangement, and any patients with day 42 MRD ≥1% (regardless of provisional classification) or persistent MRD during the consolidation phase were classified as having high-risk ALL. The remaining patients, including those with TCF3–PBX1, hypodiploidy with fewer than 44 chromosomes, T-ALL, testicular leukemia, or a CNS-3 status (>25 leukocytes/μL of cerebrospinal fluid with blasts or cranial palsies) at diagnosis were considered to have standard-risk ALL.

Transcriptome Sequencing (RNA-seq)

RNA-seq was performed on 502 samples using TrueSeq library preparation and HiSeq 2000/2500 or NovaSeq 6000 sequencers (Illumina). All sequence reads were paired-end, and sequencing was performed using (35) total RNA and stranded RNA-seq [100 base-pair (bp) reads] or (36) polyA-selected mRNA (100 bp reads). Sequencing reads were mapped to the GRCh37 human genome reference by STAR (ref. 1; version 2.4.2a) through the suggested two-pass mapping pipeline. Gene annotation downloaded from the Ensembl website (http://www.ensembl.org/) was used for STAR mapping and the following read-count evaluation. All the samples were sequenced with RedSeq2 Biocorridor R package (42). To evaluate the digital gene-expression levels, regularized log-transformed (rlog) values were calculated by DESeq2 (Supplementary Table S7). All patients received triple intrathecal chemotherapy for CNS-directed treatment with the number of doses based on the CNS characteristics and CNS status (Supplementary Table S7). Allogeneic hematopoietic cell transplantation was an option for patients with high-risk leukemia.

Main Outcomes and Measures

The primary objective of the study was to determine the prognostic and therapeutic implications of leukemia subtypes, especially the novel subtypes, among patients who had comprehensive genomic analyses and sequential MRD determination during remission induction for risk-directed treatment.

Statistical Analysis

The primary endpoint was event-free survival, and secondary endpoints were overall survival and cumulative risk of relapse. Event-free survival was defined as the time from diagnosis of ALL until the date of induction failure (≥5% blasts in bone marrow), relapse, death in remission from any cause, the development of a second cancer, or the date of last contact (all event-free survivors). Event-free and overall survival rates were estimated by the Kaplan-Meier method and compared by the log-rank test. Cumulative risk of relapse was estimated according to the method of Kalbfleisch and Prentice (45) and compared with Gray’s test (46); death in remission and the development of secondary neoplasms were regarded as competing events. The 95% CI was computed by using the asymptotic normality approximation; a nonparametric method was applied if the sample size was small. All reported P values were two-sided and not adjusted for multiple comparisons. MRD levels at each time point were categorized into three groups (<0.01%, 0.01%–1%, and ≥1%) and regarded as unordered in the analysis. Outcome data updated on June 2, 2020, were used in all analyses; 88.7% of the survivors had been seen within 1 year. The median follow-up time for the 557 patients who were alive at the time of analysis was 7 years (interquartile range 5 years; range, 1.1–7.2 years). All statistical analyses were based on intent to treat and done with SAS software (version 9.4) and R version 3.3.0.

Authors’ Disclosures

S. Jeha reports grants from NIH and other support from American Lebanese Syrian Associated Charities (ALSAC) during the conduct of the study. E. Coustan-Smith reports patent US 9,777,332 issued. H. Inaba reports grants and personal fees from Servier, personal fees from Jazz, and grants from Amgen and Incyte outside the submitted work. J.J. Yang reports grants from NIH during the conduct of the study, as well as a patent for Method and Kit for Determining Benefit of Chemotherapy pending. C. Cheng reports grants from NIH during the conduct of the study. M.V. Relling reports grants from Servier during the conduct of the study. D. Campana reports grants from NIH during the conduct of the study; other support from Juno Therapeutics, Nkarta Therapeutics, Medisix Therapeutics, and Unum Therapeutics outside the submitted work; and patent US 9,777,332 issued. C.G. Mullighan reports personal fees from Illumina during the conduct of the study, as well as grants from AbbVie and Pfizer outside the submitted work. C.-H. Pui reports other support from Adaptive Biotechnology, Inc. during the conduct of the study, as well as personal fees from Novartis, Amgen, and Erytech outside the submitted work. No disclosures were reported by the other authors.
Disclaimer

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Acknowledgments

This study was supported by NIH grants P30 CA021765, CA36401, CA176063, CA250418, CA241452, P50 GM115279, GM118578, and R35 CA197695 and the American Lebanese Syrian Associated Charities (ALSAC).

Received December 17, 2020; revised March 4, 2021; accepted April 3, 2021; published first April 10, 2021.

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Leukemia Subtypes and MRD in Childhood ALL

Clinical Significance of Novel Subtypes of Acute Lymphoblastic Leukemia in the Context of Minimal Residual Disease–Directed Therapy


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Access the most recent version of this article at:
doi: 10.1158/2643-3230.BCD-20-0229

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