MINIMAL RESIDUAL DISEASE-GUIDED THERAPY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Dario Campana1 and Ching-Hon Pui2

1Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 2Departments of Oncology and Pathology, St Jude Children’s Research Hospital, and the University of Tennessee Health Science Center, Memphis, Tennessee

Running title: Impact of MRD in childhood ALL
CASE PRESENTATION

Case 1: A 3-year old boy presented with a leukocyte count of 5.9 x 10^9/L and was found to have near-haploid B-lineage acute lymphoblastic leukemia (ALL) with a 27, X, +Y, +13, +18, +21 karyotype. He was enrolled in the St. Jude Children’s Research Hospital Total XV study. After 19 days of remission induction therapy with one high-dose methotrexate, 14 days of prednisone, 2 weekly doses of vincristine and daunorubicin, and 6 thrice-weekly doses of Escherichia coli-derived asparaginase, flow cytometry examination of his bone marrow revealed the presence of minimal residual disease (MRD) amounting to 3 leukemic cells per 10,000 mononucleated cells (0.03%). Upon completion of the remaining remission induction therapy consisting of one dose of cyclophosphamide, 14 days of mercaptopurine and 8 daily doses of cytarabine, he attained a morphologic remission on day 46, with undetectable (<0.01%) MRD by flow cytometry and polymerase chain reaction (PCR). Because of the near-haploid ALL karyotype and negative day 46 MRD, he was assigned to receive intensive chemotherapy for 3 years. MRD remained undetectable throughout treatment. He has remained in continuous complete remission for 11.6 years.

Case 2: A 9-year-old boy presented with a 3-month history of progressive pallor and upper respiratory tract infection. He had no hepatosplenomegaly or mediastinal mass. An abnormal blood count with hemoglobin 3.4 g/dL, leukocytes 4.2 x 10^9/L with 15% neutrophils, 52% lymphocytes and 33% blasts, and platelets 123 x 10^9/L prompted a bone marrow examination which disclosed 96% replacement with leukemic lymphoblasts. By flow cytometry, the blasts expressed CD45, surface CD3, CD2, CD7, T-cell receptor gamma/delta, CD11b, and CD13, with a subset of cells positive for CD56, a cell profile indicative of T-ALL. He was enrolled on
the Total XV study and began remission induction therapy with high-dose methotrexate followed by daily prednisone, weekly vincristine, weekly daunorubicin, and thrice weekly Escherichia coli-derived asparaginase. On day 19 of treatment, 62.9% of bone marrow mononucleated cells were leukemic lymphoblasts by flow cytometry (31% of all cells were blasts by morphology). Three additional doses of asparaginase were given, and the remaining remission induction therapy consisted of cyclophosphamide, mercaptopurine and cytarabine. On day 46, he attained morphological remission (with 3% lymphoblasts) but MRD by flow cytometry was 5.82%. After consolidation treatment with 4 courses of high-dose methotrexate plus mercaptopurine as well as one course of reintensification therapy with dexamethasone, etoposide, high-dose cytarabine and asparaginase, MRD decreased to 0.18%. He attained MRD-negative status (<0.01%) after a second course of reintensification treatment, and subsequently underwent a matched-related allogeneic hematopoietic stem cell transplantation (HSCT). He has remained in continuous complete remission for 11.9 years.

INTRODUCTION

The first report of minimal (i.e., not morphologically evident) residual disease (MRD) in leukemia was published nearly 4 decades ago.1 By identifying leukemic cells with fluorochrome-conjugated antisera and fluorescence microscopy, this study disclosed their presence in the bone marrow of patients with ALL after remission induction therapy. Thus, a fundamental concept in the modern evaluation and management of acute leukemia was introduced: a bone marrow in complete remission may contain leukemic cells detectable by methods that are more sensitive and objective than morphologic examination.

The initial microscopic methods were subsequently replaced by flow cytometry, and the
number and quality of antibodies available for leukemia immunophenotyping progressively increased. The expanding knowledge about the marker profile of leukemic cells together with improvements in technology led to flow cytometric methods that can identify a distinctive leukemia-associated immunophenotype in virtually all patients with ALL, and can reliably detect 1 leukemic cell among 10,000 or greater normal bone marrow or peripheral blood cells. In parallel, an impressive development has occurred for molecular methods to detect MRD in ALL, which target clonal rearrangements of immunoglobulin and/or T-cell receptor genes. By amplifying these unique molecular signatures using patient-specific PCR primers or, as shown more recently, by subjecting PCR-amplified DNA fragments of these genes to deep-sequencing analysis, MRD at levels of 1 in 100,000 or greater can be detected. The application of flow cytometry and PCR to monitor MRD in patients with ALL consolidated the notion that residual leukemia can be present at varied levels during treatment among patients who are in clinical complete remission without morphologically evident disease. Some patients achieve MRD-negative status, typically defined as <0.01% leukemic cells in bone marrow and peripheral blood, after remission induction therapy. Other patients harbor MRD at levels that can range from 0.01% to 5% or more.

Pediatric oncologists treating children and adolescents with ALL have pioneered the use of MRD to monitor response to treatment, and all major pediatric oncology centers and cooperative groups worldwide now systematically use MRD to guide treatment decisions (Table 1). Because precise measurements of MRD have important prognostic and therapeutic implications, it is essential to understand their clinical significance in the context of presenting clinical and biologic features, treatment regimen, and time interval at which MRD is measured.
MRD-DIRECTED TREATMENT OF HIGH-RISK GENETIC SUBTYPES OF ALL

(CASE 1)

Genetic abnormalities of leukemic lymphoblasts have prognostic significance and have been used to inform treatment decisions.\textsuperscript{21} The genetic subtype defined by hypodiploidy (<44 chromosomes), especially near-haploidy (24-31 chromosomes) and low-hypodiploidy (32-39 chromosomes), has generally been associated with unfavorable prognosis.\textsuperscript{22} Hence, HSCT in first remission is still offered to patients with hypodiploid (<44 chromosomes) ALL in many contemporary clinical trials.\textsuperscript{23} However, treatment outcome of hypodiploid ALL and many other high-risk genetic subtypes of ALL is not uniformly poor because it depends on other leukemia cell variables (e.g., cooperative genomic abnormalities, self-renewal capacity, drug resistance), host factors (e.g., pharmacogenetics), and efficacy of post-remission treatment regimen.\textsuperscript{21}

Our Total Therapy Studies XV and XVI relied on MRD measurements for final risk-assignment, an approach that may override or lessen the prognostic impact of specific genetic abnormality of leukemic cells.\textsuperscript{17,24} Our assumption was that sequential MRD monitoring during remission induction therapy should detect heterogeneity in chemosensitivity of leukemia cells among patients with the same genetic subtype. If so, it should be possible to use this information to adjust treatment intensity and avoid over- or under-treatment. Accordingly, patients with hypodiploid ALL were prospectively assigned to receive intensive chemotherapy, and only those with MRD \( \geq 1\% \) at the end of remission induction were offered allogeneic HSCT as a treatment option. This strategy resulted in a 5-year event-free survival of 73.6\% for the 20 patients with hypodiploid ALL, and a 5-year event-free survival of 91.7\% for the 13 who achieved MRD-negative status at the end of remission induction and were treated only with chemotherapy.\textsuperscript{22} These data demonstrate that patients with hypodiploid ALL who have a good response to
remission induction therapy as indicated by achievement of MRD negativity can be successfully treated with intensive chemotherapy alone. In our study, there were too few hypodiploid patients with positive MRD at the end of remission induction to conclusively determine if allogeneic HSCT can improve their outcome.

In the era of MRD-directed therapy, further studies, preferably randomized, are needed to identify the optimal way to incorporate MRD monitoring into treatment strategies for hypodiploid ALL and other high-risk genetic subtypes of ALL, such as Philadelphia chromosome (BCR-ABL1)-positive, Philadelphia chromosome-like, t(17;19) with TCF3-HLF fusion, and intrachromosomal amplification of chromosome 21. \(^{21}\) In the EsPhALL and Children’s Oncology Group AALL0031 studies for Philadelphia chromosome-positive ALL, treatment with intensive chemotherapy plus imatinib or allogeneic HSCT yielded comparable treatment outcomes. \(^{25,26}\) Our data indicate that the addition of ABL-tyrosine kinase inhibitor to remission induction chemotherapy in patients with this leukemia subtype can dramatically reduce the level of MRD at the end of remission induction. \(^{27}\) Thus, HSCT in first remission is warranted only for patients with Philadelphia chromosome-positive ALL who have positive MRD after intensive remission induction that includes an ABL tyrosine kinase inhibitor. In a recent retrospective study, we found that MRD-directed treatment used in our Total Therapy Study XV also improved outcome for patients with Philadelphia chromosome-like ALL. \(^{28}\) In this trial, patients with high MRD at the end of remission induction received allogeneic HSCT, while those who achieved MRD-negative status at the end of remission induction (approximately 40%) received relatively low-intensity chemotherapy and had 5-year event-free survival of 100%. \(^{28}\) Anecdotal studies suggest that the addition of ABL-class inhibitor (e.g., imatinib) can improve outcome of patients with Philadelphia chromosome-like ALL and ABL-class fusion who have
poor response to remission induction.29-32

**Recommendation.** For children and adolescents with high-risk genetic subtypes of ALL who attain MRD-negativity (<0.01%) at the end of remission induction, we recommend to proceed with intensive chemotherapy, with the addition of an appropriate tyrosine kinase inhibitor in patients with Philadelphia chromosome-positive ALL or Philadelphia chromosome-like ALL with ABL-class fusion transcript.33 As discussed below, more studies are needed to determine if allogeneic HSCT or emerging experimental therapies can benefit patients with high MRD after remission induction treatment or persistent disease after consolidation treatment.

**SPECIFIC LEUKEMIA SUBTYPES REMAIN PROGNOSTIC IN THE CONTEXT OF MRD-DIRECTED TREATMENT (CASE 2)**

Children and adolescents with ALL who do not achieve morphologic remission after the initial 4-week course of chemotherapy (“induction failure”) have been regarded to have chemoresistant disease and to be candidates for allogeneic HSCT. However, an international collaborative study showed that the prognostic impact of induction failure after conventional remission induction therapy was not uniform among different subtypes of ALL. Thus, despite induction failure, children with hyperdiploid (>50 chromosomes) B-ALL had a relatively favorable 10-year survival of 71% ± 6% when treated with chemotherapy alone, without transplantation.20 This outcome was possibly due to the known increased sensitivity of the such blast cells to methotrexate and mercaptopurine, drugs that are generally used at low doses or not at all during remission induction but are used in high doses later.20 Post-remission chemotherapy was generally not as effective in patients with other subtypes of ALL; in those with T-cell ALL
and induction failure, allogeneic HSCT was more effective than intensive chemotherapy alone.\textsuperscript{20}

Case 2 described here had T-ALL, with residual disease by flow cytometry of 62.9\% on day 19 and 5.82\% on day 46 of remission induction therapy. Because of poor early response to initial chemotherapy, he received allogeneic HSCT after achieving MRD-negative status with further chemotherapy. The AIEOP-BFM-ALL 2000 study used MRD levels on day 33 and day 78 of treatment for risk classification.\textsuperscript{34} It found that the latter measurement was more informative for predicting relapse in T-ALL, with 21\% of patients meeting their high-risk criteria of MRD \(\geq 0.1\%\) on day 78.\textsuperscript{34} These patients had a 7-year event-free survival of only 49.8\%, significantly worse than that of patients with lower levels of MRD, particularly those with MRD <0.01\% on day 33. In our Total XV study, even among patients with negative MRD on day 46, those with T-cell ALL had a poorer event-free survival (78.7\%) and an inferior overall survival (86.4\%) than did patients with other leukemia subtypes.\textsuperscript{19}

With the increasing optimization of standard therapy and the availability of new agents for ALL, an ever more refined risk algorithm combining presenting biologic and genetic features with MRD measurements is needed to develop optimal post-remission treatment strategies.\textsuperscript{19} We have shown that among patients with positive MRD at the end of remission induction, serial monitoring of MRD is important, as some patients may be cured with chemotherapy alone if MRD becomes undetectable after subsequent treatment.\textsuperscript{19} For example, in early T-cell precursor (ETP) ALL, which is generally associated with high levels of MRD during and at the end of remission induction therapy,\textsuperscript{35} recent studies suggest that post-remission chemotherapy, such as consolidation treatment phase 1B of AIEOP-BFM regimen with 2 courses of cyclophosphamide, mercaptopurine and cytarabine, might be effective in reducing MRD and mitigate adverse prognosis.\textsuperscript{36,37} In this regard, MRD measured at latter time points (e.g., day 78 of AIEOP-BFM
studies or week 14 of UKALL studies, Table 1) should be particularly useful to identify patients who have a high risk of relapse, i.e., those who have residual disease after receiving adequate doses of most, if not all, potentially effective chemotherapeutic or targeted drugs.\textsuperscript{19}

**Recommendation.** Children and adolescents with ALL who have high levels (≥1\%) MRD at the end of remission induction therapy and persistent MRD at subsequent time points have a very high risk of relapse if treated with intensive chemotherapy alone. For these patients, we recommend allogeneic HSCT. Because MRD levels before transplant are directly associated with risk of relapse post-transplant,\textsuperscript{38} additional treatment directed at reducing levels of MRD prior to transplant should be considered. Some patients with high levels of MRD at the end of remission induction, e.g., those with hyperdiploid (>50 chromosome)- or ETP-ALL, may continue treatment with intensive chemotherapy without allogeneic HSCT if they achieve MRD negative status after consolidation treatment.

**CONCLUSIONS**

MRD monitoring has redefined remission in ALL. Numerous studies have demonstrated the strong association between MRD levels and treatment outcome in childhood ALL,\textsuperscript{5,6} supporting the concept that MRD during the initial phases of chemotherapy provides a reliable measurement of the drug sensitivity of leukemic lymphoblasts. This realization has profoundly refined risk-directed therapy, with MRD being applied in virtually all major protocols for pediatric ALL to guide treatment decisions.

Table 1 summarizes some of the risk classification guidelines used in current pediatric ALL trials in the US and Europe. It is evident that there is no consensus on the precise time
points at which MRD should be measured and on the levels used for treatment decisions. The algorithms are typically built on the experience of previous correlative studies by each study group, with the timing for MRD studies adapted to the treatment design and schedule in each individual protocol. The predictive value of MRD depends on the preceding and subsequent treatment, and must be determined in the context of each treatment regimen. There are, however, some general principles that can be extrapolated from the published data, and are exemplified by the cases discussed here. Patients who have high levels of MRD (i.e., $\geq 1\%$) at the end of remission induction therapy and persistent MRD after subsequent consolidation treatment, have a very high risk of relapse if treated with currently available chemotherapy. The best treatment option for these patients is currently allogeneic HSCT, particularly if levels of MRD can be reduced to undetectable status before transplant. Emergent immunotherapeutics might facilitate MRD reduction in these patients, and could also be curative without further treatment.\textsuperscript{39,40} Conversely, patients with high-risk presenting features can be cured with chemotherapy alone if they achieve MRD negativity (i.e., $<0.01\%$) at the end of remission induction or consolidation therapy, with the possible exception of those with the t(17;19) with $TCF3$-$HLF$ fusion. To this end, MRD-guided therapy can improve the outcome of some high-risk groups of patients, such as older adolescents\textsuperscript{41} and those with hypodiploidy,\textsuperscript{22} or Philadelphia chromosome-like ALL.\textsuperscript{28}

The use of MRD in ALL relies on highly sophisticated methods and a detailed understanding of its clinical significance, evolving over 4 decades of basic, translational and clinical research. Conceivably, newer methods that can detect MRD at lower levels than the standard threshold of 0.01\% should further refine monitoring of treatment response.\textsuperscript{7,42} With 5-year survival rates exceeding 90\% in many developed countries,\textsuperscript{33} current efforts focus on the early identification of patients with highly curable leukemia, to avoid short-term morbidity and
mortality, and long-term treatment-related sequelae. In this regard, attainment of negative MRD after exposure to only a few drugs for a short duration of time (i.e., 2 weeks from treatment initiation), is a very useful indicator. This approach is particularly helpful in patients with t(12;21)/(ETV6-RUNX1) or hyperdiploid (>50 chromosomes) ALL. However, its effectiveness depends on the intensity of subsequent treatment. The risk features and MRD time points to be used must be selected with caution. Thus, in a recent analysis of the AIEOP-BFM 2000 study, an increased relapse rate was observed for patients with B-ALL regarded as standard-risk (defined primarily by leukocyte count and age) who had received reduced intensity delayed intensification because of negative MRD on days 33 and 78. With an expanding arsenal of agents for ALL, the application of MRD must be adapted so that novel treatment strategies can be designed effectively. Thus, MRD monitoring can contribute to the development of novel immunotherapeutic approaches by serving as an eligibility or response criterion.

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AUTHORSHIP

Contribution: D.C. and C-H.P. were responsible for the literature search and data collection, analysis and critical interpretation of the results, and wrote the manuscript.

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CORRESPONDENCE

Dario Campana, Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Center of Translational Medicine, 14 Medical Drive, Singapore 117599; email: paedc@nus.edu.sg

Ching-Hon Pui, Department of Oncology, St Jude Children’s Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678; e-mail: ching-hon.pui@stjude.org
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Table 1. MRD stratification for patients with B-lineage ALL in selected contemporary clinical trials

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<thead>
<tr>
<th>Study</th>
<th>Time and Site of MRD Study</th>
<th>MRD Stratification</th>
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<tbody>
<tr>
<td>AIEOP-BFM ALL 20098,9</td>
<td>Day 15 bone marrow Day 33 bone marrow Day 79 bone marrow</td>
<td><strong>SR</strong> No HR factors and either Day 33 or Day 79 MRD negative by PCR, or PCR-MRD on Day 33 or Day 79 not available and Day 15 &lt; 0.1% by FCM</td>
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<tr>
<td></td>
<td></td>
<td><strong>MR</strong> Others</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>HR</strong> HR factors, or Day 15 ≥ 10% by FCM or Day 79 ≥ 0.05% by PCR, or for B-lineage only, Day 33 ≥ 0.05% and Day 79 positive &lt; 0.05% by PCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>VHR</strong></td>
</tr>
<tr>
<td>COG AALL 08B110</td>
<td>Day 8 blood Day 29 bone marrow</td>
<td><strong>LR</strong> NCI SR Favorable genetics No unfavorable factors Day 8 &lt; 0.01% Day 29 &lt; 0.01%</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>AR</strong> NCI SR Favorable genetics No unfavorable factors Day 8 ≥ 0.01% Day 29 &lt; 0.01%, or Neutral genetics No unfavorable factors Day 8 ≥ 1% Day 29 &lt; 0.01%</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>HR</strong> NCI SR Favorable genetics No unfavorable factors Day 29 ≥ 0.01%</td>
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<td></td>
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<td><strong>NCI SR or HR Unfavorable factors</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>NCI SR Neutral genetics Day 29 ≥ 0.01%</strong></td>
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<tr>
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<td></td>
<td><strong>NCI HR Day 29 ≥ 0.01%, or Age ≥ 13 years</strong></td>
</tr>
<tr>
<td>DCOG ALL-1012</td>
<td>Day 33 bone marrow Day 79 bone marrow</td>
<td><strong>SR</strong> No unfavorable factors Day 33 and Day 79</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>MR</strong> Others</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>HR</strong> Unfavorable factors Day 33 ≥ 0.05%</td>
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<tr>
<td></td>
<td></td>
<td><strong>Induction failure Day 29 &gt;5%</strong></td>
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| Abbreviations: AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; ALL, acute lymphoblastic leukemia; BFM, Berlin-Frankfurt-Münster; COG, Children’s Oncology Group; DCOG, Dutch Children’s Oncology Group; MRC UKALL, Medical Research Council United Kingdom Acute Lymphoblastic Leukemia; SJCRH, St. Jude Children’s Research Hospital; SR, standard risk; MR, medium risk; HR, high risk; LR, low risk; AR, average risk; VHR, very high risk; IR, intermediate risk; NCI, National Cancer Institute; PCR, polymerase chain reaction; FCM, flow cytometry. In AIEOP-BFM ALL 2009, high-risk factors include prednisone poor-response, non-remission on day 33, positive *MLL-AF4* fusion or t (4;11), hypodiploidy <45 chromosomes; in COG AALL08B1, favorable genetics include double trisomy 4 and 10 or *ETV6-RUNX1* fusion, and unfavorable factors include CNS3 status, testicular leukemia, hypodiploidy <44 chromosomes or DNA index <0.81, intrachromosomal amplification of chromosome 21, M3 marrow on day 29, *MLL* rearrangement, *BCR-ABL1* fusion; in COG AALL08B1, NCI HR indicates patients with B-lineage ALL with presenting leukocyte count ≥ 50,000/μL or age ≥ 10 years and NCI SR patients without these features; in DCOG ALL-10, unfavorable factors include *MLL-AF4* infusion, prednisone-poor response, CNS3 status, testicular leukemia, and no complete remission on day 33; in NCRI UKALL 2011, unfavorable factors include *MLL* rearrangement, near haploidy <30 chromosomes, low hypoploidy 30-39 chromosomes, t (17;19) (q23;p13) and intrachromosomal amplification of chromosome 21; in SJCRH Total XVI, favorable factors include NCI SR, positive *ETV6-RUNX1* fusion, and DNA index ≥ 1.16, and unfavorable factors include CNS3 status, testicular leukemia, *BCR-ABL1* fusion, *E2A-PBX1* fusion, *MLL* rearrangement, hypodiploidy <44 chromosomes. |}
Minimal residual disease-guided therapy in childhood acute lymphoblastic leukemia

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