Adult Low-Hypodiploid Acute B-Lymphoblastic Leukemia With IKZF3 Deletion and TP53 Mutation

Comparison With Pediatric Patients

Min Fang, MD, PhD,1-3 Pamela S. Becker, MD, PhD,1,2 Michael Linenberger, MD,1,2 Keith D. Eaton, MD, PhD,1,3 Frederick R. Appelbaum, MD,1,3 ZoAnn Dreyer, MD,4 Gladstone Airewele, MD, Michel Redell, MD, PhD,4 Dolores Lopez-Terrada, MD, PhD,4 Ankita Patel, PhD,5 Karen R. Rabin,4 and Xinyan Lu, MD6

From the 1Fred Hutchinson Cancer Research Center, Seattle, WA; 2University of Washington, Seattle; 3Seattle Cancer Care Alliance, Seattle, WA; 4Texas Children’s Cancer and Hematology Centers and Department of Pediatrics, Baylor College of Medicine, Houston; 5Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; and 6Department of Hematopathology, University of Texas MD Anderson Cancer Center, Houston.

Key Words: B-cell lymphoblastic leukemia; TP53; IKZF; copy-neutral loss of heterozygosity; single-nucleotide polymorphism array; chromosome genomic array testing

ABSTRACT

Objectives: Chromosomal ploidy is a major risk stratification tool for acute B-cell lymphoblastic leukemia (B-ALL). Low hypodiploidy and near-haploidy are thought to be confined to pediatric B-ALL and associated with a poor prognosis. Doubling of either a low-hypodiploid or a near-haploid clone results in an apparently high-hyperdiploid karyotype, which is often misclassified for risk.

Methods: We studied four patients with B-ALL who had chromosome genomic array testing (CGAT), along with fluorescence in situ hybridization and mutation testing.

Results: We identified a unique case of adult B-ALL with masked low hypodiploidy (mLH) by genomic duplication, along with a somatic deletion of the IKZF3 gene and a somatic TP53 mutation. Three cases of pediatric B-ALL with mLH, two with TP53 mutations and one untested, were also identified and compared with the adult patient.

Conclusions: CGAT was critical in the genotype clarification of these cases through detection of copy-neutral loss of heterozygosity and should be considered performing for B-ALL with apparent hyperdiploidy for accurate prognostic risk stratification and treatment planning.

Chromosome number, or ploidy, in leukemic blasts plays an important role in prognostic assessment and risk stratification in patients with B-cell acute lymphoblastic leukemia (B-ALL), especially those without the translocations of t(9;22)(q34;q11.2), t(v;11q23)―MLL rearrangement, t(12;21)(p13;q22), t(1;19)(q23;p13), and t(5;14) (q31;q32) defined by the World Health Organization 2008 classification.1 Hyperdiploidy with a chromosome number greater than 50 confers a favorable prognosis.2 Near-diploid cases with 44 to 45 chromosomes often harbor dicentric chromosomes, such as dic(9;20)(p11-13;q11), or other known rearrangements, such as ETV6-RUNX1 fusion in t(12;21)(p13;q22), which are not associated with an adverse outcome. Hypodiploid acute lymphoblastic leukemia (ALL) with chromosome numbers less than 44, however, is associated with a poor prognosis.3 Three subtypes of hypodiploid ALL have been characterized in pediatric B-ALL: (1) high hypodiploidy with 40 to 43 chromosomes, (2) low hypodiploidy (LH) with 32 to 39 chromosomes, and (3) near-haploidy (NH) with 24 to 31 chromosomes.4 The hypodiploid genome may further undergo endoduplication,5,6 resulting in an apparent hyperdiploid karyotype, known as “masked hypodiploid ALL,” including both masked low hypodiploidy (mLH) and masked near-haploidy (mNH). Patients with mLH and mNH B-ALL have an extremely poor prognosis. Interestingly, LH and NH are rare in adults; NH and mNH are considered pediatric specific with virtually no adult cases reported.2 The cause of such difference observed between adults and children remains unknown.
Recent genomic profiling and sequencing efforts have led to new insights into B-ALL pathobiology and, specifically, the genomic landscape of hypodiploid B-ALL. Holmfeldt et al7 found NH ALL cases to be enriched in mutations in genes involved in receptor tyrosine kinase (RTK) and Ras signaling and in IKZF3, which encodes the lymphoid transcription factor AIOLOS. By contrast, LH ALL often harbored mutations in TP53, IKZF2, encoding HELIOS, and RB1 genes. Both NH and LH ALL demonstrated activation of the Ras and PI3K signaling pathways and sensitivity to PI3K inhibitors. Although 117 adult ALL cases were included in the study by Holmfeldt et al,7 only 11 (9.4%) were LH, and none were NH or mNH compared with 124 childhood hypodiploid ALL cases, including 50 (40%) NH cases, 26 LH cases, and masked cases in both categories.

Chromosome genomic array testing (CGAT) combines the advantageous features of both array-based comparative genome hybridization for clean copy number data and the single-nucleotide polymorphism array (SNP-A) for allelic information. Because conventional G-banding cytogenetics cannot reliably identify masked hypodiploid B-ALL cases except by highly experienced cancer cytogeneticists, CGAT can provide critical adjunctive genomic information for correct risk stratification and optimal management. In fact, a proficiency testing survey conducted in 2009 by the College of American Pathologists on 262 participating Clinical Laboratory Improvement Amendments (CLIA)–certified cytogenetics laboratories demonstrated that 54.2% of the participants failed to recognize the poor prognosis associated with the entity of NH ALL, even with a near-haploid fluorescence in situ hybridization (FISH) image presented along with the karyotype.8

Herein, we present a unique case of adult B-ALL with a mLH karyotype, a 1.7-Mb deletion encompassing the IKZF3 gene, and a TP53 mutation. To our knowledge, this is the first reported adult case of mLH with a TP53 mutation that has been successfully treated with chimeric antigen receptor T-cell (CAR-T) cell therapy. We compare this case with three pediatric B-ALL cases that also relied on SNP-based array testing to confirm the genotype of mLH, highlighting the utility of CGAT in the diagnostic workup of B-ALL. Since TP53 mutations were identified in all tested cases, this genetic lesion may be a common underlying characteristic of mLH.

Materials and Methods

Patient Information

Clinical information was obtained with institutional review board approval from both the Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA) and the Texas Children’s Cancer and Hematology Centers at Baylor College of Medicine (Houston, TX). Appropriate consent was obtained from the patients or the patients’ parents. Cytogenetics, FISH, and molecular testing were performed as part of the routine clinical workup. The results were obtained from the medical record. The adult case was the only patient with mLH from a total of 185 patients with B-ALL encountered at the Seattle Cancer Care Alliance Laboratory, FHCRC, between 2008 and 2012. Similarly, the three pediatric patients were all the mLH B-ALL cases diagnosed at the Texas Children’s Cytogenetics Laboratory from 2004 to 2010 of approximately 550 patients with B-ALL tested.

CGAT

Genomic DNA was extracted using the Gentra Puregene Blood Kit (Qiagen, Germantown, MD). Adult DNA was processed using the CytoScanHD kit from Affymetrix (Santa Clara, CA) according to the manufacturer’s protocol. Pediatric DNA was assayed using Infinium Cyto12 from Illumina (San Diego, CA). Data were analyzed with both the manufacturers’ software (ChAS from Affymetrix and Genome Studio from Illumina) and the Nexus software from BioDiscovery (Hawthorne, CA) with a clinically validated analysis algorithm. Results are described per International System for Human Cytogenetic Nomenclature (ISCN) 2013.9

Sequencing Analysis

Detailed sequencing methods have been described elsewhere for the adult case10 and the pediatric cases.11 In brief, libraries were prepared with Sure-SelectXT Target Enrichment for Illumina multiplexed sequencing. The adapter-ligated library was polymerase chain reaction amplified and quantified. Individual paired-end libraries (500 ng) were hybridized to a custom design of complementary RNA biotinylated oligonucleotides targeting multiple genes, including TP53. DNA sequencing was performed on HiSeq2000 (Illumina) with 2 × 101–base pair paired-end reads according to the manufacturer’s instructions. Data analysis tools included the Burrows-Wheeler aligner for read mapping, sequence alignment/map tools for alignment processing, and GATK Universal Genotyper (Broad Institute, Cambridge, MA) and VarScan (Washington University, St Louis, MO) for single nucleotide variant and indel calling using default parameters.

Results

Adult B-ALL Case

The patient was a 46-year-old man who presented with severe fatigue, dyspnea on exertion, iliac and sternal pain,
and low-grade fevers. The initial CBC showed a WBC count of 2.7 × 10^9/L, an absolute neutrophil count of 0.51 × 10^9/L, hematocrit value of 34%, and platelet count of 50 × 10^9/L, and 8% blasts were noted on peripheral smear. Lactate dehydrogenase was 1,583 IU/L. Testicular ultrasound showed multiple right-sided solid and hypoechoic testicular masses. Initial bone marrow contained 94% blasts by both morphologic differential and flow cytometry analysis. The immunophenotype confirmed pre-B-ALL, with blasts showing abnormal expression of CD10, decreased CD38, and normal expression of CD45, increased CD58, and normal expression of CD19 with variable CD20. He was treated initially with R-hyperCVAD (rituximab, cyclophosphamide, doxorubicin, vincristine, and dexamethasone) and intrathecal chemotherapy and after cycle 1A, there was persistent leukemia representing 23% by morphology and 20.9% by flow cytometry. He then received cycle 1B of R-hyperCVAD (high doses of methotrexate and cytarabine), and there was persistent minimal residual disease (MRD), shown as abnormal blasts at the 1% level by morphology and 2.5% by flow cytometry. He then received one cycle of rituximab, ifosfamide, mitoxantrone, and etoposide and was referred for enrollment in an experimental trial of CD19-specific CAR-T therapy. However, T-cell harvest was delayed by disease relapse, evident by 62% blasts detected by flow cytometry. He received another cycle of hyperCVAD-B with rituximab (Rituxan), which reduced his blasts to 10% by flow cytometry. After CAR-T treatment with clofarabine conditioning, he achieved a complete remission without detectable MRD by flow cytometry and molecular methods (Adaptive Biotechnologies, Seattle, WA). He then underwent allogeneic hematopoietic stem cell transplantation from his human leukocyte antigen identical sibling after myeloablative conditioning. He remains without evidence of recurrent disease at 13 months after initial diagnosis and 220 days after allogeneic transplantation.

At diagnosis, G-banding cytogenetics on the bone marrow showed an apparently high-hyperdiploid clone described per ISCN2013 as follows: 65,XY,+X,+Y,+1,+1,+2,+4,+5,+8,+10,+11,+12,+14,+15,+18,+18,+21,+21,+22,+22[cp4]/46,XY[3]. Based on this result, the patient was initially assessed to be in a favorable prognosis subgroup. FISH study on the bone marrow was negative for CDKN2A (p16) deletion, BCR/ABL1, or MLL gene rearrangement but positive for gain of chromosomes 11 and 22, consistent with cytogenetic findings. The FISH results are summarized per ISCN2013 as follows: nuc ish(CDKN2A,CEP9)+2[200],[AML1×2,ASS×2,BCR×3-5][77/200], (MLL×3)[76/200].

CGAT analysis was then carried out and demonstrated a male genotype with multiple copy number aberrations as well as copy-neutral loss of heterozygosity (cnLOH).

**Figure 1A**, as described in the following:

\[
\text{arr[hg19]}(X,Y,3,7,9,13,15,16,17,19)\times2 \text{ hinz, (1,18,20,21,22)\times4, (2,4,5,8,10,11,12,14)\times3, (6)\times2-3, 17q12(37,914,473-38,015,923)\times0-1 \text{ in which the “×2 hinz” represents two homozygous copies of chromosomes (cnLOH), whereas “×3” or “×4” represented a copy number increase and “×0-1” represented a deletion of one or both copies of the DNA segment with the indicated genomic coordinates per human genome build 19 (hg19). These results suggested that the main clonal abnormality was the doubling of a LH clone with a 101-kb IKZF3 gene deletion at 17q12.}
\]

**Figure 1B**. This case
was therefore reclassified as a rare mLH clone, which was known to be associated with a very poor prognosis in pediatric B-ALL populations. Given this information, the original G-banding karyotype was revised to 65(29<1n>, XY,+1,+18,+20,+21,+22)×2,+2,+4,+5,+6,+8,+10,+11,+12,+14[cp4]/46,XY[3].

Of note, the gain of chromosome 6 was obviously a subclone, or evolving clone, because the scale of its copy number increase was smaller than those for other three-copy (trisomic) chromosomes. The allelic track also showed a consistent result in that the split of the heterozygous track was less than that for the other chromosomes (Figure 1A).

Molecular testing was also performed using targeted sequencing on an Illumina HiSeq for detecting single-nucleotide variants, insertion, and deletions. A deleterious mutation of the TP53 gene (NM_000546.5 c.673-2A>G) was found. Because the entire chromosome 17 displayed cnLOH (Figure 1B), both the TP53 mutation and the IKZF3 deletion were homozygous in nature in the leukemia cells. No PAX5, RAS, RB1, or other RTK signaling mutations were found.

CGAT testing was repeated on the post–second induction recovery peripheral blood 3.5 months after the initial diagnosis and confirmed the absence of clonal abnormalities of cnLOH and IKZF3 deletion to the sensitivity limit of the test (20%; data not shown). Peripheral blood differential showed 1% blasts. Bone marrow was not evaluated at that time.

Three Pediatric ALL Cases With mLH

Three apparently hyperdiploid pediatric pre-B-ALL cases were also found to have mLH after SNP-A analyses. None of the three patients showed any deletion of the IKZF genes. The clinical and pathologic information, along with cytogenetics, FISH, and array findings, is summarized in Table 1 in comparison with the adult case described above. Representative FISH results are shown in Figure 2B. Two of the patients had a family history of Li-Fraumeni syndrome and were both positive for TP53 mutations. The identification of the TP53 mutation in the family of patient 1 by exome sequencing was previously reported. This patient was also identified to have the “double hits” on the TP53 mutations. Due to their reclassification as LH by CGAT testing, all three patients were switched to a more intensified chemotherapy regimen, followed by allogeneic hematopoietic stem cell transplantation. Two of the patients unfortunately died of nonleukemia complications (see Table 1 for more details).

Discussion

LH is a rare entity in B-ALL and is associated with a poor prognosis. The subtype of mNH has been considered to occur only in pediatric patients and is associated with a dismal outcome, with an event-free survival of only 30% at 3 years after diagnosis. More than half of these cases also harbor a second clone with doubling of either the LH or NH clone, which would appear as a high hyperdiploid clone. In some patients, the original NH or LH clone may be lost, leaving only the high hyperdiploid clone, which is therefore called a masked clone (mLH or mNH). Fortunately, new genomic technologies allow for the better characterization of these entities, although they are not commonly available or routinely used. Our adult patient represents, to our knowledge, the first reported case of an adult pre-B-ALL with mLH along with TP53 mutation and IKZF3 deletion. He is also among the first cohort of patients treated with the novel experimental CAR-T infusion therapy with success despite several initial treatment failures. The comparison with three pediatric pre-B-ALL cases, all of which showed mLH, highlights the similarities in genomic pathogenesis and challenges for accurate diagnosis.

In all cases presented here, CGAT (or SNP-A–based analyses) played a critical role in ascertaining the nature of the chromosomal ploidy. The allelic track information helped to determine the actual two-copy and four-copy levels of the copy number track. The loss of heterozygosity (LOH) information confirmed the doubling mechanism of the mLH clone, as well as the homozygous nature of the TP53 mutations detected (and the IKZF deletion in the adult case), even when the tumor burden was relatively low (Figures 1 and 2). Although FISH or flow cytometry to determine DNA content can also help with the detection of LH if the original NH or LH clone is present, they are of limited value when only the doubled-up clone is present. Experienced cytogeneticists can distinguish mLH from high hyperdiploidy based on the pattern of chromosomal gains. Typically, an mLH clone includes four copies of the sex chromosome and chromosome 1, 14, 18, or 22 but only two copies of chromosomes 7 and 17, whereas a high-hyperdiploid clone is generally trisomic for X, 4, 10, 17, and 18 but tetrasomic for 14 and 21. However, even among CLIA-certified cytogenetics laboratories, most failed to make the correct diagnosis and risk assessment. Therefore, CGAT or SNP-A is indispensable to confirm the mLH clone. This was also recently demonstrated by studies of pediatric B-ALL diagnosis and classification.

The reported prevalence of mNH or mLH in the literature might be underrepresented due to the limitation of previous technologies. Adult mNH cases appear to be extremely rare, with only a handful of published reports, some of which were not from the English literature. More broad application of the new genomic technologies will likely detect
more such cases. Some of the adult LH/near-triploid ALL cases reported previously may indeed be mNH or mLH, even though the authors specifically stated no patients with a NH clone were detected in these cohorts except one in the 15- to 55-year age group. Among the pediatric cases shown here, it is striking how similar the genomic plots are between patients 1 and 2 (Figure 2B). In comparison, the adult case and pediatric cases all showed duplication and LOH of chromosomes 3, 7, 9, 13, 15, 16, and 17 but never LOH for chromosomes 18, 21, and 22 (Figures 1 and 2). This similarity confirms the previous observation that the chromosomes lost and retained in the low-haploid clone are nonrandom, although the mechanism of this preference remains unknown.

Three of the four patients in this report who were tested for TP53 mutations harbored the mutations. We hypothesize that there might be an association of TP53 mutation with LH in B-ALL. This is consistent with two recent abstracts reporting a very high TP53 mutation frequency (92%) in adult patients with LH B-ALL. These cases rarely harbored IKZF1 deletion, t(9;22), or t(12;21) but showed an association with MYC rearrangement and complex karyotype. TP53 mutations and deletions do predict poor outcome in pediatric ALL. Association of TP53 mutation with adult mLH, however, is a new concept. Given the few patients with mLH available from our patient population, we acknowledge that a selection bias cannot be entirely excluded, even though these were all the patients with mLH identified in our laboratories.
| Table 1  
Clinical Information on Patients With Masked Low-Hypodiploid B-ALL |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td><strong>Pediatric Patient 1</strong></td>
<td><strong>Pediatric Patient 2</strong></td>
<td><strong>Pediatric Patient 3</strong></td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>Pre-B-ALL</td>
<td>Pre-B-ALL</td>
<td>Pre-B-ALL</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>14</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td><strong>Initial presentation</strong></td>
<td>Fever, malaise, weight loss, hepatosplenomegaly, and abdominal pain</td>
<td>Fever, abdominal pain, anemia, thrombocytopenia, and circulating blasts</td>
<td>Leg pain, fatigue, decreased appetite with weight loss, bleeding, and bruising</td>
</tr>
<tr>
<td><strong>Marrow blast, %</strong></td>
<td>50</td>
<td>36</td>
<td>75</td>
</tr>
<tr>
<td><strong>Immunophenotype</strong></td>
<td>CD10, CD19, CD20, CD22, HA-LR, CD99, CD38, and CD55</td>
<td>CD10, CD19, CD20, CD22, CD62, CD38, CD58, CD99 (partial), and HA-LR</td>
<td>CD19, CD20 (partial), CD22 (partial), HA-LR, CD62, CD99, CD38, and CD34</td>
</tr>
<tr>
<td><strong>Peripheral blood</strong></td>
<td>WBC 4.21 × 10^9/L, Hb 11.1 g/dL, platelets 71 × 10^9/L, 18.3% seg, 8.7% band, 35.6% lymph, 7.8% mono, 0.9% eos, 3.5% meta, 4.3% myelo, 20.9% blasts</td>
<td>WBC 9.4 g/dL, platelets 32 × 10^9/L, 13.0% seg, 9.6% band, 62.7% lymph, 4.3% mono, 1.7% meta, 0.9% myelo, 7.8% blasts</td>
<td>WBC 5.87 × 10^9/L, Hb 9.1 g/dL, platelets 12 × 10^9/L, 5.8% seg, 7.5% band, 11.7% lymph, 3.3% meta, 1.7% myelo, 70.0% blasts</td>
</tr>
<tr>
<td><strong>Karyotype</strong></td>
<td>66,XY,+X,+1,+3,+5,+6,+6,+8,+8,+10,+11,+1,14,+14,+18,+18,+19,+19,+21,+21,+22,+22,inc[3]; revised per CGAT: 66(31,XY,+1,+6,+11,+18,+19,+21,+22)x2,+5,+8,+10,+14,inc[3]</td>
<td>67,XX,+X,+1,+1,+5,+5,+6,+6,+8,+8,+10,+11,+13,+14,+18,+18,+19,+19,+21,+21,+22,+22,inc[3]; revised per CGAT: 67-77(13,XX,+1,+6,+8,+11,+14,+18,+19,+21,+22)x2,+5,+10,+14,inc[3]</td>
<td>36,XY,+3,+4,+5,+7,+9,+12,+13,+15,+16,+17,+20(63&lt;2n&gt;,XY)+X,1–12,+12,+14,1,2,6,+10,+11,+11,+12,+14,19,22,22[cp4]/46,XY[3]; revised per CGAT: 65-68(29,XY,+1,+18,20,21,22)x2,2,4,5,6,7,8,9,10,11,12,14,14,15,16,17,18,19,22,22[cp4]/46,XY[3]</td>
</tr>
<tr>
<td><strong>FISH</strong></td>
<td>3-4 copies for chromosomes 1, 4, 8, 10, 11, 14, 18, 19, 21, 22; 2 copies for 4, 9, 12, 17</td>
<td>3-4 copies for chromosomes X, 5, 8, 10, 11, 13, 21, 22; 2 copies of 4, 9, 12, 17</td>
<td>3-4 copies for chromosomes 10, 11, 12, 21, 22; 2 copies of 4, 10, 17, CDKN2A (9p) deletion</td>
</tr>
<tr>
<td><strong>CGAT</strong></td>
<td>Doubling of low hypodiploidy in 50% cells</td>
<td>Doubling of low hypodiploidy in 70% cells</td>
<td>Two clones showing the low hypodiploidy and doubled hypodiploid</td>
</tr>
<tr>
<td><strong>Family history</strong></td>
<td>Li Fraumeni syndrome</td>
<td>Li Fraumeni syndrome</td>
<td>No family history of cancer</td>
</tr>
<tr>
<td><strong>TP53 mutation</strong></td>
<td>c.916C&gt;T (p.R306X)</td>
<td>c.743G&gt;A (p.R248Q)</td>
<td>Not tested</td>
</tr>
<tr>
<td><strong>Chemo</strong></td>
<td>Initially treated on COG high-risk ALL chemotherapy regimen AALL0232; changed to very high-risk regimen AALL0331 when found to have hypodiploid ALL</td>
<td>Initially treated on COG high-risk ALL regimen AALL0232; changed to individualized intensified chemotherapy due to hypodiploidy</td>
<td>Initially treated on COG high-risk regimen AALL0232; changed to very high-risk regimen AALL0331 when found to have hypodiploid ALL</td>
</tr>
<tr>
<td><strong>Transplantation</strong></td>
<td>Haplod-identical sibling donor peripheral blood stem cell transplant; two donor lymphocyte infusions for mixed chimerism</td>
<td>Matched sibling donor transplant</td>
<td>Matched sibling donor bone marrow transplant</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Status</strong></td>
<td>First remission</td>
<td>First remission</td>
<td>First remission</td>
</tr>
<tr>
<td><strong>Chimerism</strong></td>
<td>100% donor type</td>
<td>100% donor type</td>
<td>100% donor type</td>
</tr>
<tr>
<td><strong>Last follow-up</strong></td>
<td>October 2013</td>
<td>February 2011</td>
<td>January 2011</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td>No</td>
<td>Yes due to chronic skin and liver GVHD, liver and renal failure, hemorrhagic cystitis secondary to JC virus infection, and secondary engraftment failure shortly prior to death. No evidence of recurrent leukemia at time of death</td>
<td>Yes due to veno-occlusive disease of the liver</td>
</tr>
</tbody>
</table>

| **Overall survival, mo** | 22 (alive) | 8.6 | 13 (alive) |

**ALL**, acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CAR-T, chimeric antigen receptor T cell; CGAT, chromosome genomic array testing; COG, Children’s Oncology Group; eos, eosinophils; FISH, fluorescence in situ hybridization; GVHD, graft vs host disease; Hb, hemoglobin; hyperCVAD-B, part B of cyclophosphamide, doxorubicin, vincristine, and dexamethasone combination therapy; JC virus, John Cunningham virus; lymph, lymphocytes; meta, metamyelocytes; mono, monocytes; myelo, myelocytes; R-hyperCVAD, rituximab, cyclophosphamide, doxorubicin, vincristine, and dexamethasone; seg, segmented neutrophils.
In pediatric B-ALL, NH is characterized by a high frequency of activating mutations of Ras signaling (mainly NF1 alterations), IKZF3 alterations, and mutations targeting the RTK signaling, whereas LH is characterized by IKZF2, TP53, and RB1 alterations.\(^7\) Our finding of mLH, TP53 mutation, and IKZF3 deletion in an adult patient is truly unique. There were no RAS, RB1, or other RTK signaling mutations found in this patient.

IKAROS family DNA-binding zinc finger transcription factors have gained much recognition for their roles in hematologic malignancies since the initial description of IKZF1 alterations in Philadelphia chromosome–positive ALL.\(^{23,24}\) IKZF1 regulates the development and function of the immune system and acts as a master regulator of normal hematopoietic differentiation and proliferation, particularly in lymphoid lineages.\(^{25,26}\) None of the three pediatric cases reported here showed any evidence of IKAROS family gene copy number alterations. Knockdown of Ikzf2 and Ikzf3 in mouse pre–B-cell lines showed an increase in both pERK and pS6, suggesting that HELIOS and AIOLOS, protein products of IKZF2 and IKZF3, respectively, may also modulate Ras and PI3K signaling in addition to lymphoid cell development.\(^{27}\) The IKZF3 deletion shown in our adult patient is somatic and not present in the remission sample. AIOLOS has various splicing isoforms, which heterodimerize with IKAROS and associate with histone deacetylase-containing complexes to affect histone modification and target binding.\(^{28}\)

In summary, we report four mLH B-ALL cases detected by CGAT, one adult and three pediatric. This is a rare entity with a very poor prognosis, and SNP-based microarray testing is critical for the correct diagnosis to avoid misclassification. The association between TP53 mutation and mLH in adult patients with B-ALL is intriguing and requires additional cases to firmly establish this link.

Corresponding author: Min Fang, MD, PhD, Fred Hutchinson Cancer Research Center, 825 Eastlake Ave E, G7-500, Seattle, WA 98109-1023; mfang@fhcrc.org.

Support provided by Clinical Research Division, Fred Hutchinson Cancer Research Center.

References


