B-Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

Sanam Loghavi, MD,1 Jeffery L. Kutok, MD, PhD,2 and Jeffrey L. Jorgensen, MD, PhD1

From the 1Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston; and 2Infinity Pharmaceuticals, Cambridge, MA.

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ABSTRACT

Objectives: This session of the 2013 Society of Hematopathology/European Association for Haematopathology Workshop was dedicated to B-acute lymphoblastic leukemia (B-ALL)/lymphoblastic lymphoma (LBL) with recurrent translocations and not otherwise specified.

Methods: In this review, we summarize the cases discussed during the workshop, review the pertinent and most recent literature on the respective topics, and provide a few key points that may aid in the workup of patients with B-ALL/LBL.

Results: Many of the submitted cases showed interesting diagnostic, immunophenotypic, or clinical aspects of B-ALL with BCR/ABL1, MLL-associated, and other recurrent chromosomal abnormalities. Several cases showed rare aberrancies such as coexistent IGH/BCL2 and MYC rearrangements and raised issues in classification. Other cases had unusual clinical presentations, including B-ALL with hypereosinophilia and therapy-related B-ALL. Several cases highlighted the role of flow cytometry immunophenotyping in distinguishing benign B-cell precursors from aberrant lymphoblasts, and other cases raised questions regarding the clinical importance of myeloperoxidase positivity in acute lymphoblastic leukemia.

Conclusions: The complexity and spectrum of cases presented in this review highlight the importance of clinicopathologic correlation and the value of ancillary studies in the classification and workup of patients with B-ALL/LBL.

B-acute lymphoblastic leukemia (B-ALL)/lymphoblastic lymphoma (LBL) is a neoplasm of immature B-cell precursors that typically affects children younger than 6 years but is also encountered in older children and in adult populations. The estimated global incidence of B-ALL is around one to five per 100,000 persons per year.1 Patients with B-ALL usually have signs and symptoms of bone marrow (BM) failure, including cytopenias with or without leukocytosis. The diagnosis is established by immunophenotyping, commonly by flow cytometry (FC), which shows immature B lineage. Many cases of B-ALL harbor recurrent chromosomal abnormalities, including balanced chromosomal translocations, which are often critical determinants of prognosis.

B-ALL With Recurrent Cytogenetic Abnormalities

What Are the Features of Philadelphia Chromosome–Positive B-ALL?

Approximately 20% to 30% of adult acute lymphoblastic leukemias (ALLs) and 5% of pediatric ALLs harbor the Philadelphia (Ph) chromosome. This genetic alteration confers a poor prognosis, as defined by shorter remission duration and shorter survival and higher rates of resistance to standard chemotherapy.2-4 Allogeneic stem cell transplant in first remission is a common therapeutic option. The Ph chromosome is the result of t(9;22)(q34;q11.2) that creates a BCR-ABL1 fusion gene residing on a minute derivative chromosome 22. The fusion gene encodes a constitutively...
active BCR-ABL1 tyrosine kinase, which in turn promotes unregulated cell proliferation.

Case 164 was an example of a patient with Ph+ B-ALL (BCR-ABL1; p190), refractory to induction chemotherapy, who achieved complete remission with dasatinib, a second-generation tyrosine kinase inhibitor (TKI). Targeted therapy with TKIs in combination with conventional chemotherapy has dramatically improved the outcome of patients with Ph+ B-ALL. The addition of the first-generation TKI, imatinib, to standard chemotherapy regimens has substantially increased complete remission rates in patients with B-ALL; however, up to 30% of patients are either initially resistant or eventually go on to develop resistance to imatinib, most commonly as a result of BCR-ABL kinase domain mutations. Alternatively, imatinib resistance has also been attributed to the Src family of kinases that are involved in ALL leukemogenesis but are unresponsive to imatinib. The e1a2 (p190) fusion transcript, as seen in patient 164, has been shown to be associated with unfavorable response to imatinib therapy with short-lived responses in the setting of chronic myelogenous leukemia (CML). Dasatinib, a dual Src/Abl kinase inhibitor, has shown clinical efficacy in inducing substantially better hematologic and cytogenetic remission in patients with Ph+ ALL with imatinib resistance. The superior effect of dasatinib may be attributed to its increased potency, its ability to inhibit mutant variants of BCR-ABL, and/or its capability to target Src family kinases.

Three additional patients with Ph+ B-ALL (cases 109, 314, and 335) were discussed. Ph+ B-ALL blasts often show high expression levels of CD10 (uniform, bright), CD11b, CD13, CD15, CD25, and CD66c, as well as partial or complete lack of CD34 expression. Case 314 was a child with Down syndrome (DS) and B-ALL. Apart from t(8;14)(q11;q32), which is significantly overrepresented in DS children with B-ALL, the incidence of recurrent chromosomal abnormalities associated with B-ALL, including t(9;22), is significantly lower in this patient population. The lower frequency of unfavorable recurrent cytogenetic abnormalities may account for the better clinical outcome in this population.

Case 210 was a patient with recurrent Ph+ B-ALL with an IKZF1 deletion. The IKZF1 gene encodes for Ikaros, a protein involved in B-cell differentiation and proliferation, presumably by modulating immunoglobulin heavy chain gene (IGH) rearrangement and cell signaling in immature B-cell receptors. Wild-type Ikaros has been shown to harbor tumor suppressor activity. Partial or complete deletions of IKZF1 affecting the gene start codon on exon 2 lead to haploinsufficiency, while deletions of DNA-binding domains on exons 4 to 7 result in loss of tumor suppressor activity via their dominant negative effect on the unaffected allele. Recent studies have shown that IKZF1 deletions are independent poor prognostic indicators in BCR-ABL1–negative as well as BCR-ABL1–positive B-ALL in both pediatric and adult patients. IKZF1 deletions are significantly more common (~70%) in Ph+ B-ALL compared with BCR-ABL1–negative disease. Mullighan et al identified a high frequency of IKZF1 copy number alterations as well as deleterious mutations of IKZF1 in Ph+ ALL and CML in blast phase but not in chronic phase CML. Their findings suggest that Ikaros alterations are directly linked to leukemogenesis in Ph+ ALL. In a pediatric-based study by van der Veer et al., the negative effect of IKZF1 deletion in Ph+ B-ALL persisted even in patients who were treated with TKIs, irrespective of whether they received hematopoietic stem cell transplant. The authors suggested that IKZF1 be used in further risk stratification and therapy planning in patients with BCR-ABL1–positive B-ALL. Martinelli et al. showed similar effects in adults with Ph+ B-ALL. Using gene expression profiling techniques, recent studies have shown evidence of activated Bruton tyrosine kinase (BTK) and JAK-STAT pathways in B-ALL with IKZF1 deletion, suggesting a potential role for targeted therapy with BTK and JAK inhibitors in this patient population. What Are the Features of B-ALL With Associated MLL Alterations?

Six cases (118, 203, 235, 272, 386, and 415) submitted to this session were examples of de novo B-ALL with MLL (11q23) gene rearrangements. More than 75 gene partners for MLL have been identified. B-ALL associated with MLL gene rearrangements represents a distinctive group with characteristic clinical findings, gene expression signature, and immunophenotypic and prognostic features. Patients with these tumors have a bimodal age distribution with high prevalence among infants younger than 1 year of age and an increasing incidence in later adulthood, while being relatively uncommon in older children. Approximately 20% of all cases of ALL, including 80% of infant ALL and 10% of older children and adult ALL, exhibit MLL rearrangements. Both infants and adults with B-ALL associated with MLL rearrangements have a poor prognosis. The t(4;11)(q21;q23) with an AFD fusion partner is most common in infants, but other fusion partners also can be seen in infants (cases 118 and 386), and t(4;11)(q21;q23) also can be observed in adults (case 272).

Most B-ALL cases with MLL alterations show a characteristic aberrant immunophenotype with absence of CD10 and expression of the myeloid markers CD15, CD33, and/or CD65. This pattern was seen in four of six cases in this session, with one tumor (case 386) showing partial positivity for CD10. Two cases showed unusual
### Table 1
Summary of Cases Submitted to the 2013 Society of Hematopathology/European Association for Haematopathology Workshop: B-Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Interesting Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>164</td>
<td>B-ALL, Ph+ (BCR/ABL1; p190)</td>
<td>Refractory to ALL induction therapy but CR with dasatinib</td>
</tr>
<tr>
<td>109</td>
<td>B-ALL, Ph+ (BCR/ABL1; p210)</td>
<td>Characteristic phenotype: CD10++, CD34+, CD13 dim+, CD38+</td>
</tr>
<tr>
<td>314</td>
<td>B-ALL with Ph+ in DS</td>
<td>Ph+ is rare in DS; CD10 subset+, CD13+, CD34+</td>
</tr>
<tr>
<td>335</td>
<td>B-ALL with Ph+ and complex karyotype (monotypic surface light chain)</td>
<td>CD10++, CD19+, CD20 dim+, CD33 dim+, CD34 partial+, CD45 dim+, TdT+, sIg dim+</td>
</tr>
<tr>
<td>210</td>
<td>Recurrent Ph+ B-ALL, with IKZF1, EBF1, and PAX5 deletion</td>
<td>SNP array; IKZF1 deletion associated with poor prognosis</td>
</tr>
<tr>
<td>272</td>
<td>B-ALL with t(4;11)(q23;q32)/MLL rearranged</td>
<td>Adult, MLL-associated phenotype (CD10−, CD19+, HLA-DR+ by flow cytometry; PAX5+ and CD79a+ by IHC)</td>
</tr>
<tr>
<td>203</td>
<td>B-ALL with 46,XX,ins(11;19)(q32;p13.3p13.3)(MLL+,MLLT1+;MLLT1+)/MLL rearranged</td>
<td>A cryptic insertional translocation resulting in MLLT1/MLL fusion demonstrated by FISH; MLL-associated phenotype (CD10−)</td>
</tr>
<tr>
<td>235</td>
<td>B-ALL with MLL gene rearrangement</td>
<td>Adult presentation; MLL-associated phenotype (CD19+, CD10−, CD15+)</td>
</tr>
<tr>
<td>386</td>
<td>Congenital B-ALL with t(11;19)(q23;p13.3)/MLL rearranged</td>
<td>38-week old with MLL-associated phenotype but subset CD10+, not the typical t(4;11) seen in infants</td>
</tr>
<tr>
<td>118</td>
<td>B-ALL with AF9-MLL fusion (t9;11)</td>
<td>Infant with mature B-cell phenotype with surface λ light chains; not typical t(4;11) seen in infants</td>
</tr>
<tr>
<td>415</td>
<td>Mature B-cell leukemia/lymphoma with t(4;11)</td>
<td>Child with mature B-cell phenotype with slgM λ, negative for CD10, CD20, CD34, and TdT</td>
</tr>
<tr>
<td>203</td>
<td>B-ALL with t(4;11)(q23;q32)/MLL rearranged</td>
<td>Adult, MLL-associated phenotype (CD10−, CD19+, HLA-DR+ by flow cytometry; PAX5+ and CD79a+ by IHC)</td>
</tr>
<tr>
<td>371</td>
<td>B-ALL with eosinophilia and IGH gene rearrangement by FISH</td>
<td>Marked eosinophilia; FISH negative for abnormalities in PDGFR, PDGFRB, FGFR1, MLL, IL-3, and BCR-ABL1 fusion</td>
</tr>
<tr>
<td>102</td>
<td>Concurrent B-ALL and myeloproliferative neoplasm, not otherwise specified</td>
<td>1p uniparental disomy (UPD1p) as well as MPL (p.W515S) gene mutation</td>
</tr>
<tr>
<td>111</td>
<td>B-ALL with a hypodiploid karyotype</td>
<td>Masked hypodiploid karyotype identified by SNP array</td>
</tr>
<tr>
<td>104</td>
<td>B-ALL with t(5;14)(q31;q32)</td>
<td>Eosinophilia; 47,XY,(5;14)(q31;q32),+22; PDGFRB not involved</td>
</tr>
<tr>
<td>132</td>
<td>B-ALL with t(5;14)(q31;q32)</td>
<td>Eosinophilia; 47,XX,(5;14)(q31;q32) with additional 20q–</td>
</tr>
<tr>
<td>371</td>
<td>B-ALL with eosinophilia and IGH gene rearrangement by FISH</td>
<td>Eosinophilia; FISH for IGH rearrangement; FIP1L1-PDGFRα, TCRβ and γ rearrangements negative</td>
</tr>
<tr>
<td>387</td>
<td>B-ALL with hypereosinophilia</td>
<td>Marked eosinophilia; FISH negative for abnormalities in PDGFR, PDGFRB, FGFR1, MLL, IL-3, and BCR-ABL1 fusion</td>
</tr>
<tr>
<td>149</td>
<td>B-ALL with t(8;22)(q11.2;q11.2)</td>
<td>Widespread lymphadenopathy, sheets of BM blasts; CD10+, CD20 dim+, TdT+, CD34–slg–; small mature subset, CD20 bright slg+, CD10-TdT−</td>
</tr>
<tr>
<td>301</td>
<td>High-grade TdT+ blastic B-cell leukemia/lymphoma with t(3;13)(q27;q14)</td>
<td>Possible transformation of follicular lymphoma</td>
</tr>
<tr>
<td>336</td>
<td>High-grade TdT+ blastic B-cell leukemia/lymphoma with t(14;18)</td>
<td>Abdominal mass, sheets of BM blasts; CD10+, CD19+, TdT+, CD20−, CD34+, slg–</td>
</tr>
<tr>
<td>369</td>
<td>High-grade TdT+ blastic B-cell leukemia/lymphoma with BCL2 (18q21) and MYC (8q24) translocations</td>
<td>Abdominal mass, sheets of BM blasts; CD10+, CD19+, TdT+, CD20−, CD34+, slg–, high SSC</td>
</tr>
<tr>
<td>149</td>
<td>B-ALL with t(8;22)(q11.2;q11.2)</td>
<td>Retroperitoneal lymphadenopathy, sheets of BM blasts; CD10+, CD19+, TdT+, CD20−, CD34−, slg–, high SSC</td>
</tr>
<tr>
<td>321</td>
<td>B-ALL</td>
<td>Gene expression profiling; high FLT3 expression, “cluster group B,” associated with better prognosis</td>
</tr>
</tbody>
</table>
Table 1 (cont)

Summary of Cases Submitted to the 2013 Society of Hematopathology/European Association for Haematopathology Workshop: B-Acute Lymphoblastic Leukemia/Lymphoblastic Lymphoma

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<tbody>
<tr>
<td>142</td>
<td>Residual B-ALL</td>
<td>Aberrant phenotype by flow cytometry (4%) posttherapy, CD58 bright+</td>
</tr>
<tr>
<td>152</td>
<td>B-ALL with initial oligoblastic presentation</td>
<td>Presented with cytopenias; 6% aberrant lymphoblasts by flow cytometry; rebiopsy after 6 weeks showed 80% blasts</td>
</tr>
<tr>
<td>178</td>
<td>BM with prominent regenerating immature B-cell population</td>
<td>~50% atypical cells but benign phenotype by flow cytometry</td>
</tr>
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</table>

Significance of MPO expression in classification of acute leukemias

<table>
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<tbody>
<tr>
<td>228</td>
<td>B-ALL with t(4;11)(11q23)/MLL rearrangement</td>
<td>CD19+, CD15+, TdT++; CD10–, MPO+ by IHC, MPO– by flow cytometry</td>
</tr>
<tr>
<td>410</td>
<td>Ph+ acute leukemia, myeloid vs mixed lineage, favor myeloid</td>
<td>BM CD13+, CD19+, CD33+, CSF blasts with two subsets: CD13+, MPO+, CD19–, CD22–, TdT– vs CD13+, CD22+, TdT+, CD19+, MPO–</td>
</tr>
<tr>
<td>98</td>
<td>Therapy-related myeloid neoplasm, mixed phenotype acute leukemia (myeloid/B)</td>
<td>History of ovarian CA, cytotoxic treatment 3 years prior; blasts with complex karyotype, CD19+, CD22+, TdT+, CD10–, MPO+; subsequent relapse as AML with no B-lineage markers</td>
</tr>
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</table>

Other unusual immunophenotypic findings in B-ALL

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<thead>
<tr>
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<tbody>
<tr>
<td>128</td>
<td>B-ALL with minimal CD19 expression</td>
<td>CD10+, CD19a+, CD79a–, TdT–, MPO– by flow cytometry</td>
</tr>
<tr>
<td>260</td>
<td>B-ALL with partial immunophenotypic shift at relapse</td>
<td>Blasts at relapse lost TdT, partially lost CD10 and CD43; subset gained sIgM, sMPO</td>
</tr>
</tbody>
</table>

Therapy-related B-ALL

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<thead>
<tr>
<th>Case No.</th>
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<tbody>
<tr>
<td>297</td>
<td>Therapy-related B-ALL with t(4;11)(q21;q23)</td>
<td>Following cytotoxic therapy for breast carcinoma 1.5 years earlier</td>
</tr>
<tr>
<td>157</td>
<td>Therapy-related B-ALL</td>
<td>History of plasma cell myeloma, cytotoxic treatment 6 years ago; diploid karyotype</td>
</tr>
<tr>
<td>336</td>
<td>Therapy-related B-ALL</td>
<td>History of AML, cytotoxic therapy 5 years ago; diploid karyotype</td>
</tr>
<tr>
<td>96</td>
<td>Therapy-related B-ALL, with t(4;11)(q721; q723)/MLL rearrangement</td>
<td>History of uterine CA, DLBCL DH with cytotoxic therapy 14 years and 1 year earlier, respectively</td>
</tr>
<tr>
<td>429</td>
<td>Therapy-related abnormal population of B-lymphoid blasts</td>
<td>History of AML, cytotoxic therapy 3 years ago; 10.5% aberrant B lymphoblasts; complex karyotype with 2 subclones: 47,XY,+8,10.X[13]</td>
</tr>
</tbody>
</table>

B-ALL associated with HLH

(infection-associated hemophagocytic syndrome)

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<thead>
<tr>
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<tbody>
<tr>
<td>259</td>
<td>B-ALL in a patient with history of HLH secondary to disseminated Mycobacterium tuberculosis infection</td>
<td>B-ALL occurred months after the diagnosis of HLH</td>
</tr>
</tbody>
</table>

Novel therapy

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Interesting Features</th>
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<tbody>
<tr>
<td>264</td>
<td>Refractory chronic lymphocytic leukemia</td>
<td>Disease apparently cured by autologous CART-19</td>
</tr>
<tr>
<td>224</td>
<td>B-ALL</td>
<td>Treatment with CART-19</td>
</tr>
</tbody>
</table>

ABL1, Abi-interactor 1; AML, acute myeloblastic leukemia/lymphoma; AML, acute myeloid leukemia; B-ALL, B-acute lymphoblastic leukemia; BCR, breakpoint cluster region; BM, bone marrow; c, cytoplasmic; CA, carcinoma; CART-19, chimeric antigen receptor-modified T-cell therapy against CD19; CD, cluster of differentiation; CR, complete remission; CSF, cerebrospinal fluid; DH, double-hit; DLBCL, diffuse large B-cell lymphoma; DS, Down syndrome; EBF1, early B-cell factor 1; FIP1LI, factor interacting with PAPOLA and CPSF1; FISH, fluorescence in situ hybridization; FLT3, FMS-related tyrosine kinase 3; HLH, hemophagocytic lymphohistiocytosis; Ig, immunoglobulin; IGH, immunoglobulin heavy chain; IHC, immunohistochemistry; IKZF1, Ikaros family zinc finger 1; ins, insertion; iso, in situ hybridization; MLL, mixed-lineage leukemia gene; MLLT1, myeloid/lymphoid leukemia translocated to 1; MPL, proto-oncogene, thrombopoietin receptor; MPO, myeloperoxidase; MYC, myelocytomatosis viral oncogene homolog; Pax5, paired box gene 5; PCR, polymerase chain reaction; PDGFR, platelet-derived growth factor receptor; PDGFRB, platelet-derived growth factor receptor β; Ph, Philadelphia chromosome(9;22)(q34;q11.2); s, surface; SNP, single-nucleotide polymorphism; SSC, side scatter; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.
mature B-cell phenotypes, with expression of clonal surface light chains. One showed blastic morphology (case 118) and could be classified as B-ALL. The second case was difficult to definitively classify, since the neoplasm had Burkitt-like morphology with intermediate-sized cells containing cytoplasmic vacuoles (case 415). The review panel thought this neoplasm was best designated as a mature B-cell leukemia/lymphoma associated with t(4;11).

Additional B-ALL cases with MLL rearrangements are discussed below in the therapy-related section (cases 96 and 297) and with other neoplasms that were myeloperoxidase (MPO) positive (case 228).

**What Are the Features of B-ALL With Associated Eosinophilia?**

Four cases submitted to this session (cases 104, 132, 371, and 387) were examples of B-ALL with associated myeloid proliferations. Four of these patients had eosinophilia. In general, the presence of eosinophilia occurring with B-ALL raises several diagnostic possibilities, including but not limited to B-ALL associated with t(5;14) (q31;q32); lymphoid neoplasms with abnormalities of PDGFRA, PDGFRB, or FGFR1; and lymphoid blast crisis of CML. It is important to appropriately identify Ph+ cases and those with PDGFRA or PDGFRB abnormalities.
because these patients are often responsive to TKI therapy.51,52 Thus, in patients with nonreactive eosinophilia, in addition to conventional cytogenetic analysis, fluorescence in situ hybridization (FISH) studies are recommended to identify **FIP1L1-PDGFRA** fusions and reciprocal translocations involving **PDGFRA** (4q12), **PDGFRB** (5q31-q33), and **FGFR1** (8p11-13).53 Among the cases discussed at the workshop, two (cases 104 and 132) demonstrated the t(5;14)(q31;q32) translocation, resulting in juxtaposition of the promoter region of the interleukin-3 (**IL-3**) gene on chromosome 5 and the **IGH** gene on chromosome 14.54 In a third patient (case 371), the t(5;14)(q31;q32) was suspected and **IGH** rearrangement was detected by FISH, but conventional cytogenetics were negative for t(5;14)(q31;q32), and **IL-3** was not tested by FISH. Cases with t(5;14)(q31;q32) are rare, may affect children and adults, and have variable degrees of eosinophilia. The eosinophilic proliferation is thought to be nonclonal and results from increased IL-3 levels. The neoplastic lymphoblasts typically exhibit a precursor B-cell immunophenotype characterized by CD10 and CD19 expression. Patients with ALL are generally acutely ill and commonly have peripheral blood cytopenias (anemia and/or thrombocytopenia). In addition, the presence of any degree of eosinophilia (above high normal range) **Image 2** B-acute lymphoblastic leukemia with associated **MLL** rearrangement. The bone marrow core biopsy specimen (A) shows sheets of immature blasts. Fluorescence in situ hybridization studies (B) showed loss of a 3′ **MLL** signal (red) in 123 of 200 cells. Flow cytometry immunophenotyping (C) showed a characteristic aberrant lymphoblast phenotype, positive for CD19, CD15, and CD34 (partial) and negative for CD10. FITC, fluorescein isothiocyanate; PE, phycoerythrin; SSC, side scatter. Case 235, courtesy of J. C. Gomez-Gelvez, MD, and colleagues.)
immunophenotyping, should prompt a workup to exclude this disease entity. The t(5;14)(q31;q32) is leukemia defining, since there is no minimum required blast count to establish the diagnosis of ALL once this karyotypic abnormality is confirmed. The presence of eosinophilia in case 387 was unexplained. In this neoplasm, there was evidence of IGH rearrangement by FISH, but no abnormalities were detected in PDGFRα, PDGFRβ, FGFR1, MLL, IL-3, or BCR-ABL1 fusion.

Case 102 was unusual since this 64-year-old man had leukocytosis of 51.3 × 10⁹/L, left-shifted neutrophils, moderate monocytosis, and 2% blasts in the blood smear. The diagnosis of B-ALL was established by BM examination. Three months following chemotherapy, the patient’s BM was hypercellular with atypical megakaryocytes and clustering of megakaryocytes. The diagnosis of concurrent B-ALL and a myeloproliferative neoplasm with MPL p.W515S point mutation, suggestive of a prefibrotic phase of primary myelofibrosis, was established. Whether the B-ALL was a form of leukemic transformation of the preceding myeloproliferative neoplasm or represents a secondary malignancy is unclear.

What Is the Importance of a Hypodiploid Karyotype in B-ALL?

Hypodiploidy has unfavorable prognostic implications in patients with B-ALL, and therefore identification of hypodiploidy is important.⁵³ One case was submitted to this session. Case 11 was from a 10-year-old boy with B-ALL with a hypodiploid karyotype. The karyotype was initially thought to be a possible hyperdiploid karyotype on conventional cytogenetic studies, due to duplication of the hypodiploid clone. In this case, single-nucleotide polymorphism array analysis was performed, which unveiled the masked hypodiploid karyotype.

TdT+ B-Cell Neoplasms With BCL2 and/or MYC Alterations: What Is the Best Classification?

Five cases of TdT+ B-cell neoplasms with BCL2 rearrangements, MYC rearrangements, or both were discussed in this session. Four patients had high-grade neoplasms at presentation (cases 149, 159, 301, and 338), and the fifth patient (case 369) had a complicated clinical course that needs more explanation. This patient initially had a low-grade follicular lymphoma, followed 2 years later by high-grade B-cell lymphoma with a mature immunophenotype with FISH positive for both BCL2 and MYC rearrangements (“double hit”). One year later, the patient had a blastic TdT+ neoplasm involving BM and a retroperitoneal mass, and these were the specimens submitted to the session. Overall, four of these neoplasms showed BCL2 gene rearrangements by FISH and/or conventional karyotyping, two of which had MYC rearrangement, and a third case had BCL2, MYC, and BCL6 rearrangement (“triple hit”). One case had only a MYC rearrangement.

All patients presented in a distinctive and similar manner with large abdominal/retroperitoneal masses and replacement of the BM by sheets of blasts. The blasts in all cases were intermediate to large in size, with varying amounts of cytoplasm from case to case.
Prominent vacuoles were seen in one double-hit case and one case with only BCL2 rearrangement. All tumors were positive for CD10, CD19, and TdT; negative or only partially positive for CD34; negative or only dimly positive for CD20; and negative for surface immunoglobulins. Case 159 also had a distinct small subset of mature B cells detectable by FC, positive for CD20 and surface immunoglobulin and negative for CD10 and TdT. Immunohistochemical studies performed on the BM biopsy specimen showed a CD20+ peri- to paratrabecular infiltrate.

In case 369, there is strong evidence for transformation of a mature high-grade B-cell lymphoma to a blastic TdT+ neoplasm, with shared cytogenetic aberrations in both specimens. In case 159, there is also morphologic and immunophenotypic evidence strongly suggesting an underlying low-grade B-cell lymphoma, possibly follicular lymphoma. Blast transformation of follicular lymphoma is a rare event but has been recognized for more than 25 years. While many cases reported since have shown blast morphology but a mature immunophenotype, a few cases have shown a true precursor B-cell phenotype, positive for TdT and usually negative for CD20 and surface light chains. Most of these cases had double-hit cytogenetics, with rearrangements of both BCL2 and MYC genes. Patients with these neoplasms have a highly aggressive course, with all reported survival durations of less than 1 year.

The pathogenesis of lymphoblastic transformation of follicular lymphoma is not well understood. The t(14;18) translocation breakpoints in follicular lymphomas show evidence of recombinase and TdT activity, implying origin in an immature B-cell precursor in the BM (reviewed by Kridel et al65). In one scenario, secondary genetic transforming events take place in a residual immature lymphoma precursor cell, which retains its lymphoblastic phenotype. In an alternative model, secondary genetic events occur in mature follicular lymphoma cells, leading to “dedifferentiation” with reactivation of an immature immunophenotype. In two reported cases, the transformed lymphoblasts showed somatic hypermutation of their IGH variable region (V) sequences. These IGHV sequence changes were shared by both the original follicular lymphoma and the lymphoblasts. This finding strongly suggests that in at least some cases, follicular lymphoma may transform by dedifferentiation to a lymphoblastic phenotype.

De novo B-ALL with t(14;18)(q32;q21) has also been long recognized. Cases with a well-documented immature immunophenotype, including TdT expression, are rare. These tumors share many similarities with follicular lymphoma with lymphoblastic transformation, including frequent association with MYC translocations and a very poor prognosis. Hardianti et al76 demonstrated somatic hypermutation of IGHV gene sequences in three
TdT+ cases, at high rates comparable to follicular lymphomas. In addition, two TdT+ cases in that series showed continuing expression of activation-induced cytidine deaminase (AID). Both the presence of somatic hypermutation and AID expression are strongly associated with mature germinal center B-cell differentiation. These findings suggest a distinct pathogenetic mechanism in at least a subset of cases with t(14;18), in comparison to cases of B-ALL with more typical translocations. These cases also raise the possibility of a history of subclinical follicular lymphoma in these patients.

Overall, compared with typical B-ALL/LBL, cases of blastic transformation of follicular lymphoma and de novo lymphoblastic cases associated with t(14;18) show distinctive clinical features, including a poor prognosis, and likely a different pathogenesis. In the opinion of the consensus panel, classifying these cases as B-ALL or B-LBL without further comment could be misleading to clinicians and patients. The consensus of the review panel and participants in this session thought a descriptive diagnosis was more appropriate, such as “high-grade TdT-positive blastic B-cell leukemia/lymphoma,” and classified cases 159, 301, 338, and 369 as such. If there is a history of follicular lymphoma or evidence for underlying lower grade lymphoma, this should be clearly indicated. A history of lymphoma, extensive lymphadenopathy, or unusual immunophenotype or morphology in a TdT+ case should prompt suspicion of appropriate workup. We recommend FISH to assess for the presence of MYC and BCL2 rearrangements in such cases.

MYC gene rearrangements rarely may be observed in B-ALL/LBL in the absence of t(14;18) translocation (recently reviewed by Seo et al80). While the detection of an MYC gene rearrangement may have therapeutic implications, particularly for children on cooperative trials, the prognostic implications are not as clear. The panel classified case 149 as B-ALL with t(8;22)(q24.1;q11.2).

**B-ALL Not Otherwise Specified (Normal Karyotype)**

A single case in this category was submitted to this session. Case 321 was from a 17-year-old girl who developed B-ALL with a diploid karyotype. Of interest, the lymphoblasts were studied by gene expression profiling that showed high levels of FMS-related tyrosine kinase 3, as well as high expression of “outlier” genes. The overall profile was similar to those seen in what is known as “cluster group 5,” which has been associated with a more favorable prognosis,81 and this patient was in remission 5 years after diagnosis. This case highlights the value of gene expression profiling in diploid cases of B-ALL.

**Issues in Immunophenotyping of B-ALL**

**How Does One Distinguish B-ALL From Normal Counterparts by FC?**

Several cases in this session demonstrated the utility of FC immunophenotyping to distinguish aberrant B-ALL lymphoblasts from their benign counterparts in the BM. CD10+ B-cell precursors, termed hematogones based on their immature-appearing morphology, have a well-characterized immunophenotype notable for a spectrum of expression of several markers during maturation. Multiple studies have characterized the profile of hematogones and highlighted common immunophenotypic aberrancies associated with B lymphoblasts.81,82,83,84,85 Aberrant blasts may express antigens from an inappropriate cell lineage (eg, myeloid antigens), but it is often more informative to identify altered levels of expression of antigens that are normally expressed on hematogones.

Case 142 was from a patient with treated B-ALL who had 4% residual aberrant blasts by FC immunophenotyping on follow-up BM examination. The diagnosis was difficult, since the blast immunophenotype largely overlapped with a hematogone profile with expression of CD10, CD19, CD20, CD34, CD38, and TdT. However, the blasts did show aberrantly increased CD58 expression, demonstrating the utility of an extended minimal residual disease–targeted panel. Case 152 was from a patient who initially had cytopenias and an oligoblastic picture, with only 6% aberrant BM blasts identified by FC immunophenotyping, on the basis of decreased CD45 and increased CD10 expression. Within 6 weeks, this patient developed overt ALL with 80% blasts on a subsequent BM examination.

Case 178 was from an adult patient treated for B-ALL; follow-up BM smears showed about 50% morphologically atypical cells suspicious for residual disease [Image 51]. However, FC analysis clearly demonstrated a benign immunophenotype characteristic of regenerating hematogones. Increased hematogones may be seen in healthy children, in response to peripheral cytopenias, and in BM recovery in patients who have undergone chemotherapy or stem cell transplant.

**Does MPO Expression Occur in B-ALL, and What Is Its Importance?**

MPO can be present in rare cases of B-ALL, in contrast with the 2008 World Health Organization classification guidelines, which state that MPO expression, together with strong evidence of B-lineage differentiation, is sufficient to classify a case as mixed-phenotype acute leukemia (MPAL).86 MPO detection can be performed by cytochemistry, FC, and/or immunohistochemistry (IHC), and 3% and 10% cutoffs have been used historically as thresholds for MPO positivity by cytochemistry and FC, respectively.87,88
In a recent study, Guy et al proposed a refined threshold of 13% or 28% MPO positivity (using either isotype controls or normal lymphocytes as negative controls) to establish myeloid differentiation by FC immunophenotyping. However, MPO stains by either IHC or FC can be difficult to quantify and are prone to technical artifacts. Borowitz has recently published his opinion that MPO positivity as the sole myeloid feature in an otherwise typical ALL should be considered skeptically and may not be sufficient for a diagnosis of MPAL.

Three cases with MPO expression were discussed in this session. Case 228 was an acute leukemia with a t(4;11)(11q23) MLL rearrangement, and the blasts had a typical MLL-associated B-ALL immunophenotype (CD10–, CD15+, CD19+, TdT+). The neoplastic cells were positive for MPO by IHC (confirmed with repeat staining by the consensus panel), but MPO expression was not detected by FC immunophenotypic analysis. This discordance appears to further weaken the significance of the MPO staining, and the panel concurred with the submitting diagnosis of B-ALL.

Case 410 was a Ph+ acute leukemia that expressed several myeloid markers, including MPO (on a subset of blasts in the cerebrospinal fluid). Blasts in the BM showed strong CD19 expression but no other B-lineage markers, and those in the cerebrospinal fluid were CD22+TdT+ but CD19–. Overall, the number of concurrently expressed B-lineage markers did not appear to definitively support a diagnosis of...
MPAL. This case highlights the propensity for Ph+ leukemias to express markers from multiple lineages.

Case 98 was a therapy-related leukemia with a complex karyotype and predominantly B-antigen expression (CD19+CD22+TdT+ but CD10−) at presentation. Immunohistochemical staining showed strong coexpression of MPO, consistent with MPAL. After therapy, the patient had a recurrence with acute myeloid leukemia (AML), showing no B-lineage marker expression. It has been well established that cases of MPAL can exhibit phenotypic shift over time following therapy or at relapse and may recur as either AML or ALL.96,101-103

Other Unusual Immunophenotypic Findings in B-ALL

Case 128 was a B-ALL with a normal karyotype and an unusual immunophenotype with minimal expression of CD19 but positive for the B-lineage–associated antigens cCD79a+ and cIgM+. Reduced expression levels of CD19 may be seen in a subset of cases of B-ALL (~16%-19%),21,87,89 but significant loss of CD19 is rare. However, awareness of such cases is important because many laboratories rely on CD19 expression in the initial identification of potential B lymphoblasts.

Case 260 is an example of B-ALL with phenotypic shift at relapse, with loss of immature markers (TdT) and gain of more mature B-lineage markers (surface IgM k) on a subset. Loss and gain of antigens at relapse B-ALL is well known, with loss of CD10, CD20, and/or myeloid antigens and gain of CD20 representing the most common changes.92,104 Antigenic shifts also may manifest as altered intensity of previously expressed antigens. Several possible mechanisms have been proposed that may explain these phenomena: (a) occurrence of a second clonally unrelated leukemia; (b) presence of minor subclones at diagnosis, which are more chemoresistant than the dominant clone and become the prevailing blast populations at relapse; or (c) a phenotypic switch due to gain of additional genetic aberrancies in the course of disease progression.92

Unusual Clinical Presentations

Is Therapy-Related B-Acute Lymphoblastic Leukemia/Lymphoma a Distinct Entity?

Four cases of therapy-related ALL (t-ALL) were submitted to the workshop (cases 96, 157, 297, and 336). One patient had a history of a solid tumor (breast carcinoma), two had been treated for hematopoietic neoplasms (plasma cell myeloma and AML), and one patient had a history of both uterine carcinoma and diffuse large B-cell lymphoma. The patients all had received cytotoxic chemotherapy, starting between 1 and 6 years prior to the diagnosis of B-ALL. The patient with t-ALL after breast carcinoma had also received adjuvant radiotherapy. Two patients had t(4;11) involving the MLL locus, and both had received anthracycline-containing regimens within the preceding 12 to 18 months. The patient with myeloma received intensive chemotherapy, including alkylating agents, anthracyclines,
and etoposide, and 5 years later developed B-ALL with an aberrant karyotype, including a del(20q). The patient with AML received standard chemotherapy and 5 years later had B-ALL with a normal karyotype.

One additional patient (case 429) had a history of AML with normal cytogenetics, with relapse 3 years later. Subsequent BM examination over the next year showed persistent myeloid blasts ranging from 4% to 9% of cellularity, with a complex karyotype including clones with trisomy 8 and either isochromosome 9q or ring chromosome 9. A BM aspirate and biopsy specimen 5 years after initial therapy showed a distinct population of aberrant B-lymphoid blasts with no myeloid markers (10.5% of cells). The karyotype was complex and included aberrant clones similar to those observed in previous biopsy specimens. The overall findings suggest evolving therapy-related B-ALL but could represent persistence/recurrence of the original AML, with a lineage shift possibly due to clonal evolution.

t-ALL, defined as acute lymphoblastic leukemia arising in the setting of prior chemotherapy and/or radiation therapy, is uncommon compared with de novo disease and represents approximately 12% of all therapy-related leukemias and less than 5% of all adult acute lymphoblastic leukemias. The age of onset is older in t-ALL in comparison to de novo disease, with a reported range of 15 to 94 years. Primary sites include solid organs and the hematopoietic system. The median latency interval ranges from 19 to 72 months in various studies, depending on the clinical setting and the genetic abnormalities. Underlying dysplasia may be seen in a subset of cases, although this may be difficult to appreciate in a typical BM packed with blasts.

The most common genetic abnormalities encountered in the setting of t-ALL are t(4;11)(q21;q23) resulting in an MLL/AF4 fusion, followed by the Ph and hypodiploidy, commonly involving chromosomes 5, 7, and 17. Normal karyotypes may be seen in a minority of cases. As observed in therapy-related myeloid neoplasms, MLL rearrangements in t-ALL are most commonly seen following exposure to topoisomerase II inhibitors. Hypodiploidy is frequently associated with alkylating agents. While MLL may be seen with numerous fusion partners in both de novo ALL and in therapy-related myeloid neoplasms, this gene typically is partnered with AF4 in t-ALL, although other rare translocations involving the MLL gene have been described. Most cases of t-ALL are of B lineage, and those cases with MLL rearrangements typically show the MLL-associated immunophenotype described above. Cases of t-ALL with hypodiploidy are often positive for CD20 as well as CD10.

Patients with t-ALL have a significantly poorer outcome compared with patients with de novo disease, and the median survival of patients with t-ALL is less than 1 year. Comparison with historical controls suggests that patients with t-ALL have a worse prognosis than do adult patients with de novo B-ALL with similar cytogenetic alterations such as MLL/AF4 fusions. However, a recent comparison of patients with t-ALL and de novo ALL treated at a single institution found that karyotype but not prior cytotoxic therapy was an independent prognostic indicator in multivariate analysis. Thus, it remains an open question if t-ALL should be classified as a distinct entity or whether its distinctive clinical features are due primarily to...
its association with poor-risk cytogenetics, as well as with older age at presentation.

**B-ALL Associated With Hemophagocytic Lymphohistiocytosis**

Case 259 was from a patient with B-ALL arising in the setting of hemophagocytic lymphohistiocytosis (HLH; also known as infection-associated hemophagocytic syndrome) secondary to disseminated *Mycobacterium tuberculosis* infection. In this patient, B-ALL occurred several months after the diagnosis of HLH. This is a rare phenomenon, most commonly observed in the pediatric population. The association of HLH and ALL has been well described,\(^{114-123}\) but unlike the case submitted to the workshop, in most reported cases, the diagnosis of ALL preceded HLH. In many cases, HLH has been associated with a concurrent infectious etiology, possibly due to immune deficiency following chemotherapy or stem cell transplantation for ALL, and in these patients, the term infection-associated hemophagocytic syndrome may be more appropriate. HLH-associated ALL can be of B or T lineage, although most reported cases were T-ALL. The prognosis of these patients is often poor. If untreated, most patients with HLH will succumb to their disease within approximately 2 months. Although the diagnostic criteria for HLH are mostly clinical and laboratory dependent,\(^ {124}\) early identification of hemophagocytosis on BM examination can trigger appropriate workup and timely diagnosis, resulting in prompt immunosuppressive therapy.\(^ {124}\) Based on our review of the literature, it also seems that the term HLH is used differently by different authors, and criteria need to be refined.

**Novel Therapy**

**Chimeric Antigen Receptor-Modified T-Cell Therapy Against CD19 (CART-19)**

Chimeric antigen receptor T cells (CARTs) are genetically engineered cells, usually obtained from the patient’s peripheral blood, that express receptors capable of specifically binding to tumor-associated antigens. Chimeric antigen receptors (CARs) are fusion proteins composed of an antigen recognition moiety, usually a monoclonal antibody variable region, and costimulatory (ie, CD28 and 4-1BB) and T-cell activation domains (ie, CD3 ζ). In contrast to T-cell receptors, CARs recognize and bind to cell surface antigens and can be used to treat patients irrespective of human leukocyte antigen type (reviewed by Kochenderfer and Rosenberg\(^ {125}\)). The pan-B-cell antigen CD19 is an attractive target for using this technique in the treatment of B-cell malignancies. Kochenderfer et al\(^ {126}\) demonstrated for the first time dramatic in vivo response to anti-CD19 CARTs in patients with advanced-stage follicular lymphoma. Several clinical trials have been since conducted employing CART-19 in treating refractory B-cell lymphomas/leukemias of various subtypes, mostly in patients with chronic lymphocytic leukemia\(^ {127-129}\) (case 264), but also including a single patient with B-ALL.\(^ {130}\) Case 224 was from a patient with multiply relapsed B-ALL with a complex karyotype, successfully treated with CART-19.

**Summary**

In summary, we highlight some key points raised in the workshop and discussed in this review, with a focus on the diagnostic workup of B-ALL/LBL:

1. Deletions and other alterations in the *IKZF1* (Ikaros) gene are adverse prognostic indicators, in both Ph+ and Ph– patients with B-ALL. Particularly if targeted therapy becomes available, analysis of this locus may become important in the initial workup of B-ALL.

2. ALL associated with *MLL* gene rearrangements has a characteristic immunophenotype, CD10– with aberrant expression of CD15, that may provide the first clue to the presence of this poor-prognosis cytogenetic aberration.

3. We recommend evaluation for B-ALL in the diagnostic workup of patients with hypereosinophilia. Conventional cytogenetics, FISH, and/or polymerase chain reaction should be employed to exclude B-ALL with t(5;14)(q31;q32); lymphoid neoplasms with abnormalities of *PDGFRα*, *PDGFRβ*, or *FGFR1*; and lymphoid blast crisis of CML.

4. Consider FISH analysis for *BCL2* and *MYC* aberrancies in apparent ALL cases with extensive lymphadenopathy or in patients with a history of lymphoma. A subset of these cases may represent high-grade transformation of mature B-cell lymphomas. In cases with *BCL2* rearrangement, we do not recommend the diagnosis of B-ALL/LBL and suggest an alternative descriptive diagnosis such as high-grade TdT+ blastic B-cell leukemia/lymphoma.

5. Hematogones may be markedly increased in BM specimens during early recovery following chemotherapy or stem cell transplant in ALL. FC immunophenotyping can reliably distinguish hematogones from residual ALL blasts.

6. We advocate assessing MPO expression in all new acute leukemias by at least two techniques, including cytochemistry. In a B-ALL with an otherwise typical phenotype, MPO positivity by FC and/or IHC does not always indicate a diagnosis of MPAL.

7. Therapy-related ALL in patients with prior chemotherapy or radiation therapy is often associated with poor-prognosis cytogenetic aberrancies, frequently with t(4;11) (q21;q23)/*MLL/AF4*. It is unclear whether prior therapy is an independent poor prognostic factor.

References


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