Precursor Lymphoid Neoplasms

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Neoplastic Hematopathology Update 2017

Precursor lymphoid neoplasms

- Abnormal proliferations of immature lymphoid cells.
- Biologically and clinically heterogenous but most present as acute leukemia.
- Majority (75%) occur in children, adolescents and young adults.
- Median age at diagnosis = 15 years

The evolving classification

- French-American-British (FAB) Classification (1976)
  - Based on morphologic features of blasts on aspirate smears and cytochemistry
  - Did not include lymphoblastic lymphoma
- Flow cytometric immunophenotyping => Immunological classification of ALL
  - Pro-, Pre-pre-, pre-, mature B-cell ALL
  - Pro-, Pre-, cortical, mature T-cell ALL

I have no disclosures.
2001 WHO Classification

- Divided blastic lymphoid proliferations by lineage:
  - Precursor B-cell
  - Precursor T-cell

- Defined lymphoblastic leukemia and lymphoma as different manifestations of the same disease.

- Incorporated clinical features.

- Provided guidelines for the diagnosis of mixed lineage acute leukemia.

2001-2008: Genetic abnormalities in precursor lymphoid neoplasms

- Numerical abnormalities (additions, deletions, amplifications)
  - Reciprocal translocations leading to formation of fusion genes and expression of chimeric proteins
  - Translocations resulting in juxtaposition of a proto-oncogene next to TCR or Ig promoter gene => over-expression of normal or truncated protein

- Mutations

2008 WHO Classification

**B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities**

- Hyperdiploidy
- Hypodiploidy
- t(9;22)(q34;q11.2); BCR-ABL1
- t(v;11q23); MLL rearranged
- t(12;21)(p13;q22); TEL-AML1
- t(1;19)(q23;p13.3); E2A-PBX1
- t(5;14)(q31;q32); IL3-IGH

**B lymphoblastic leukemia/lymphoma, not otherwise specified**

**T lymphoblastic leukemia/lymphoma**

Case 1

- 26 year old Hispanic woman who presented with worsening fatigue of several months' duration, dyspnea on exertion, and easy bruising.

- CBC
  - WBC 900/uL: Hbn 8.2g/dL; platelets 44,000/uL
  - Differential: Blasts 5%, Neut 42.0%, Lymphs 53%

- Bone marrow examination
  - Hypercellular marrow (95%) with 81% blasts

- Flow cytometry:
  - CD10 (strong), CD19, CD22, CD24, CD34, CD38, CD45 (dim to negative), Tdt, and HLA-DR
Case 1

- FISH Cytogenetics: Negative for BCR/ABL1, KMT2A (MLL)
- Molecular: Negative for BCR-ABL1 p210 and p190 fusion products
- Cytogenetics: 47, XX, inv(9)(p13q34.1), +10 [11/23]
- Diagnosis: B- lymphoblastic leukemia, not otherwise specified.
- Treated with modified augmented Berlin-Frankfurt-Munster (BFM) regimen, induction cycle 1

Translocations in precursor B neoplasms

- Common: Reciprocal translocations resulting in chimeric protein expression
  - t(9;22)(q34;q11) => BCR/ABL1
  - t(1;19)(q23;p13) => TCF3-PBX1 (PBX/ETA)
  - t(4;11)(q21;q23) => KMT2A (MLL/AF)
  - t(12;21)(p13;q22) => ETV6/RUNX1 (TEL/AML1)
- Rare: Juxtaposition of a proto-oncogene to Ig promoter => over-expression
  - t(5;14)(q31;q32) => IL3/IGH

“Ph-Like B-ALL”

- Gene expression profiling studies identified a group of B-ALL lacking BCR-ABL1 but with a pattern of gene expression very similar to that seen in ALL with BCR-ABL1.
- Relatively common: 15% childhood, 30% adult B-ALL
  - 10% children with standard risk ALL
  - 13% children with high risk ALL
  - 21% adolescents
  - 27% young adults (< 40yrs)
  - 30% adults (> 40yrs)
- Males > Females
- More frequent in Hispanic and Native American populations
- Associated with a high WBC at presentation, MRD+ at end of induction, and poor outcome


The number of pathways is limited, and all may be amenable to treatment with tyrosine kinase inhibitors.


The presence of Ph-like genetic abnormality was an independent predictor of poor prognosis across all ages.

Clinical outcomes of patients with Ph-like ALL and B-other ALL. (A) OS, (B) EFS, and (C) remission duration of Ph-like ALL and B-other ALL.


2016 Classification

- B lymphoblastic leukemia/lymphoma, not otherwise specified
  - B lymphoblastic leukemia/lymphoma with
    - t(9;22)(q34;q11.2); BCR-ABL
    - t(v;11q23); KMT2A (MLL) rearrang.
    - t(12;21)(p13;q22); ETV6-RUNX1
    - Hyperdiploidy
    - Hypodiploidy
    - t(1;19)(q23;p13.3); TCF3-PBX1
    - t(5;14)(q31;q32); IGH/IL3
    - BCR-ABL1-like (provisional)
    - iAMP21 (provisional)
  - T lymphoblastic leukemia/lymphoma

Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.


<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
<th>Fusion Partner</th>
<th>Patients</th>
<th>% Cases</th>
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<tbody>
<tr>
<td>ABL1</td>
<td>Dasatinib</td>
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<td>14</td>
<td>ETY5, NAP22, ROS511, BCR-ABL1, EM22, JQ1</td>
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<td>Dasatinib</td>
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<td>FGZ08, RO5486, QMLH4</td>
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<td>PDGFRA</td>
<td>Dasatinib</td>
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<td>11</td>
<td>ENXL, SBBP2, TNP1</td>
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<td>PLZF</td>
<td>Jak2 inhibitor</td>
<td>2</td>
<td>16</td>
<td>2G4, FORT1</td>
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<tr>
<td>Jak2</td>
<td>Jak2 inhibitor</td>
<td>10</td>
<td>19</td>
<td>TDF2, BCR, QMLH4, ETV6, AML1, FRAP1, SBBP2, ETN1, TNP1, TMR2, TMR3</td>
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<td>PTK2B</td>
<td>Jak2 inhibitor</td>
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<td>JQ1, 1G8</td>
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<td>c-STAT5</td>
<td>Jak2 inhibitor</td>
<td>1</td>
<td>3</td>
<td>LMK1, ABL1</td>
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<td>IGF1R</td>
<td>Jak2 inhibitor</td>
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<td>1</td>
<td>MNK-466</td>
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<td>NT5RE</td>
<td>Chromatin</td>
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<td>2</td>
<td>ETY5</td>
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<tr>
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<td>Tyk2 inhibitor</td>
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<td>1</td>
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* The gene is a previously unrecognized fusion partner.
‡ PDGFRa-APRIL has been reported in a myeloid cancer, including angiosarcoma* and secondary breast carcinoma, but it has not previously been described in acute lymphoblastic leukemia.*

The majority of patients with Ph-like ALL have genetic alterations that respond to tyrosine kinase inhibitors that are already FDA-approved.
**Case 1**

- Comprehensive molecular profiling by next generation sequencing: **NUP214-ABL1 fusion**
  - Previously reported primarily in T-LBL (~6-8%)
  - **NUP214** (Nucleoporin 214): Located on 9q34.13
    - Nucleocytoplasmic transporter
  - **ABL1**: Located on 9q34.12
    - Tyrosine kinase
  - **NUP214-ABL1**: Results from amplification with formation of episomal elements

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  - t(5;14)(q31;q32); IGH/IL3
  - t(5;14)(q31;q32); IGH/IL3
  - **BCR-ABL1-like (provisional)**
  - **iAMP21 (provisional)**
  - **T lymphoblastic leukemia/lymphoma**

**Case 1 Follow-up**
- Day 28 marrow showed persistent blasts (25%)
- Treated with induction cycle 2 and **dasatinib**
- Persistent blasts (3%)
- Allogeneic stem cell transplantation
- Fully engrafted but minimal residual disease + post-HCT
- Treated with dasatinib maintenance

**Case 2**
- 23 year old man who presented with shortness of breath and was found to have a mediastinal mass and a large pleural effusion.
- CT-guided needle core biopsies: T lymphoblastic lymphoma
  - IHC: CD2, CD3, CD5, CD7, CD10, CD99, TdT
- Right pleural effusion: T lymphoblastic lymphoma
  - Flow Cytometry: CD1a, CD2, cytoplasmic CD3, CD4, CD5, CD7, CD8, CD10, CD34 (weak/partial), CD38, CD45, TdT
- Bone marrow: Negative
Case 2

On admission:
- Thoracentesis=> 1900 cc of bloody fluid.
- Cytology: T lymphoblastic lymphoma
- Flow cytometry: 79% blasts expressing CD1a, CD2, cytoplasmic and partial surface CD3, CD4, CD5, CD7, CD8, CD38, HLA-DR, Tdt (79% of cells)
- Treated on Phase III trial with bortezomib on a modified augmented BFM backbone

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- T lymphoblastic leukemia/lymphoma

Genetic abnormalities in precursor T neoplasms
- 50% of patients have normal karyotype at diagnosis
- Mutations are present in 60% of cases
- Translocations are present in 30% of cases
- Deletions are frequent if detected by FISH
  - Del(9)(p21) resulting in loss of the tumor suppressor gene CDKN2A in 70% => loss of G1 control in the cell cycle

Translocations in precursor T neoplasms
- Common: Juxtaposition of a proto-oncogene to TCR promoter => over-expression
  - t(10;14)(q24;q11) => HOX11
  - t(1;14)(p32-34;q11) => TAL1
  - t(7;9)(q34;q34.3) => NOTCH1
- Rare: Reciprocal translocations resulting in chimeric protein expression
  - t(11;19)(q23;p13.3) => MLL/ENL
  - ABL1 rearrangements (NUP214/ABL1)
Mutations in precursor T neoplasms

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>Activating mutations NOTCH1</td>
<td>58%</td>
</tr>
<tr>
<td>Missense mutations FBXW7</td>
<td>30%</td>
</tr>
<tr>
<td>PHF6</td>
<td>20%</td>
</tr>
<tr>
<td>OTHERS....</td>
<td></td>
</tr>
</tbody>
</table>

Spinella et al. Oncotarget 2016: 7;

Case 2: Comprehensive mutational profiling by NGS

Mutations:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide</th>
<th>Amino Acid</th>
<th>Allele Frequency (%)</th>
<th>COSMIC ID</th>
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</thead>
<tbody>
<tr>
<td>CCND1</td>
<td>c.153_154ins12</td>
<td>p.R252*</td>
<td>24.87</td>
<td>N/A</td>
</tr>
<tr>
<td>FBXW7</td>
<td>c.3065C&gt;T</td>
<td>p.R689W</td>
<td>34.36</td>
<td>27083</td>
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<tr>
<td>NOTCH1</td>
<td>c.4178T&gt;C</td>
<td>p.L1593P</td>
<td>57.31</td>
<td>13042</td>
</tr>
<tr>
<td>PHF6</td>
<td>c.8129C&gt;T</td>
<td>p.R274*</td>
<td>76.13</td>
<td>111447</td>
</tr>
</tbody>
</table>

Case 2

NOTCH1 c.4778T>C; p.L1593P
- Encodes NOTCH1 transmembrane protein
- Functions in multiple developmental processes, cell-cell interactions, receptor
- Regulates T-cell development, with a direct downstream target of MYC
- Mutations present ~60% of T lymphoblastic leukemia/lymphoma
- L1593P mutation is in the HD domain and is an activating mutation.

FBXW7 c.2065C>T; p.R689W
- Tumor suppressor gene encoding ubiquitin E3 ligase which targets for degradation several proto-oncogenes including NOTCH1
- Encodes FBXW7, a negative regulator of NOTCH1
- Missense mutation results loss of function => decreased proteasomal degradation of NOTCH1=> increased half-life of NOTCH1 protein
- R689W previously described in in T-ALL

LY3039478 is a NOTCH1 inhibitor under study for relapsed/refractory disease with NOTCH1 or FBXW7 mutations.

PHF6 c.820C>T; p.R274*
- Encodes transcriptional co-repressor
- Mutations present in ~20% T lymphoblastic leukemia/lymphoma
- R274* occurs in the ePHD2 domain and is a common hotspot
- Nonsense alteration => loss of function

CCND3 c.753_754 ins12; p.R252*
- Encodes a protein that regulates CDK kinases in the cell cycle
- Normal protein is critical for lymphocyte development, cell cycle control, and is a downstream target of activated NOTCH-1 in T lymphoblastic leukemia/lymphoma
- In-frame 12 base pair insertion => nonsense alteration at codon 252=> loss of function of the gene.
- Not previously reported
Case 3
- 66 year old woman with leukopenia and extensive lymphadenopathy.
- CBC:
  - Hb 13.8 g/dL with normal RBC indices
  - Platelets 152,000/µL
  - WBC = 1,440/µL with 6% neutrophils, 3% bands, 69% lymphocytes, 4% monocytes, 18% blasts
- Bone Marrow:
  - Hypercellular marrow (95%) with 80% blasts
  - Negative for myeloperoxidase, Sudan black B and dual esterase by cytochemistry

Case 3
- Del (7)(q22q34) in 1/20 metaphases
- FISH: Del 7q31 in 15% nuclei
- Molecular studies for TCR gamma chain gene rearrangements demonstrated a clonal rearrangement.

Diagnosis?
Early T-cell precursor lymphoblastic leukemia

- New subtype first identified in series of childhood T-ALL
- ~12% pediatric, 5-10% adults T-ALL
- Associated with poor clinical outcome
  - 10 year OS and EFS ~20%
- Increased genomic instability
  - Complex karyotypes
  - More genomic gains and losses
- Distinctive phenotype

Early T-cell precursor lymphoblastic leukemia

- Distinctive gene expression profile similar to normal early T-cell precursors
  - Recent immigrants from bone marrow to thymus
  - Retain multi-lineage differentiation potential
- Activating mutations in cytokine receptor and RAS signaling pathways
  - NRAS, KRAS, BRAF, FLT3, IL7R, JAK3, JAK1, SH2B3
- Inactivating mutations of genes encoding hematopoietic development transcription factors
  - GATA3, ETV6, RUNX1, IKZF1, EP300
- Mutations in histone modifying genes
  - EZH2, EP300

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  - Hyperdiploidy
  - Hypodiploidy
  - t(1;19)(q23;p13.3); TCF3-PBX1
  - t(5;14)(q31;q32); IGH/IL3
  - BCR-ABL1-like (provisional)
  - iAMP21 (provisional)

- B lymphoblastic leukemia/lymphoma, not otherwise specified
- T lymphoblastic leukemia/lymphoma
- Early T-cell precursor lymphoblastic leukemia
- NK-lymphoblastic leukemia/lymphoma (provisional)

CAP/ASH Guideline: Key Question #1

- What clinical and laboratory information should be available during the initial diagnostic evaluation of a patient with acute leukemia?
  - Age, sex, ethnicity
  - History of any hematological disorder, prior malignancy, or other predisposing conditions, including family history.
  - Exposure to cytotoxic therapy, radiation, immunotherapy, toxins, etc.
  - Confounding factors: recent growth therapy, transfusions, etc.
  - Relevant physical examination and imaging findings.

CAP/ASH Guideline: Key Question #2

- What specimens and sample types should be evaluated during the initial work-up?
  - CBC with differential, peripheral blood smear
  - Bone marrow aspirate for:
    - Morphological examination
    - Cytogenetics/FISH
    - Comprehensive flow cytometry immunophenotyping
    - Molecular testing
  - Core biopsy, touch imprints, clot sections if obtained.
  - If receiving intra-thecal therapy, cerebrospinal fluid for cell count and differential, and morphological examination by pathologist.
  - If extramedullary disease without marrow or blood involvement, tissue biopsy with sufficient tissue to perform the above.
**CAP/ASH Guideline: Key Question #3**

At the time of diagnosis, what tests are required for all patients?

- Everything on the previous slide plus
- Cytochemical studies if needed for lineage assignment
- Flow cytometric studies sufficiently comprehensive to allow subsequent detection of minimal residual disease (MRD)

**CAP/ASH Guideline: Key Question #4**

Which tests should be performed on only a subset of patients, including in response to results from initial tests and morphology?

- Pediatric patients with B-LBL
  - t(12;21) ETV6-RUNX1
  - t(9;22) BCR-ABL1
  - KMT2A (MLL) rearrangement
  - iAMP21
  - Trisomy 4, trisomy 10
- Adults with B-LBL
  - t(9;22) BCR-ABL1
  - KMT2A (MLL) rearrangement
- Additional mutational analysis including
  - B-LBL: PAX5, JAK1, JAK2, IKZF1
  - T-LBL: NOTCH1, FBXW7

**CAP/ASH Guideline: Key Question #5**

Where should laboratory testing be performed?

- Laboratories in compliance with regulatory and/or accreditation requirements

For any given assay, find the lab that:
- Has expertise in the specific disorder.
- Understands the technology and uses it appropriately.
- Provides clinically useful results.
- Is cost-effective.
**CAP/ASH Guideline: Key Question #6**

Arch Pathol Lab Med 2017;141:1342-1393

- **How should test results and the diagnosis be correlated and reported?**
  - Initial report: all laboratory, morphological, immunophenotypic, cytochemical data on which diagnosis is based, AND a list of any tests pending at the time of initial signout.
  - Results of pending tests for classification, management, prognosis, and disease monitory should be added to the pathology report as these become available.
  - *Diagnosis and classification using the current WHO terminology.*