Acute Lymphoblastic Leukemia Update

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Acute Lymphoblastic Leukemia (ALL) & Lymphoblastic Lymphoma (LBL)

The precursor lymphoid neoplasms
Lymphoblastic Malignancies

- Baby B or T cells gone bad (rarely NK cells)
- Leukemia = dominant PB/BM presentation
  - Often LN, liver, spleen, involvement
  - Mediastinal mass (T-ALL) +/- pleural effusions
- Lymphoma = dominant extramedullary presentation
  - Often PB/BM involvement
  - Mediastinal mass (T-LBL) +/- pleural effusions
- Essentially the same immature cells, just in different places, so are classified together
LBL vs ALL

- Blast percentage in PB/BM determines designation
- For AML, 20% blasts = AML
- In general, for ALL alone, not sure how many blasts you need, but not recommended to call ALL with <20% blasts
- For ALL vs LBL, 25%
  - Somewhat arbitrary
  - Determined by clinical trial protocols
  - Most cases fairly obviously high or low level involvement, but some are borderline
- In children, ALL and LBL often treated on ALL type protocols
Acute Lymphoblastic Leukemia

- Primarily a disease of children
- Most cases occur between ages 2-10 years
  - 75% of cases occur in children <6 years old
- 80% cure rate in kids
- 85% of pediatric cases are B cell, 15% are T cell
  - T-cell ALL more common in older patients (classically teenagers), may present with a mediastinal mass, pleural effusions
ALL: Clinical Features

- Primarily disease of blood & bone marrow
- Extramedullary involvement:
  - CNS, lymph nodes, spleen, liver, gonads
- Present with consequences of bone marrow failure
  - Anemia, low platelets, neutropenia
  - Fatigue, bleeding/petechiae/bruising, fever/infection
- WBC may be normal, low, or high
- Lymphadenopathy, splenomegaly, hepatomegaly common
- Bone pain & arthralgias may be prominent
Classification of Acute Lymphoblastic Leukemia

- **FAB – 1970’s-80’s**
  - L1, L2, L3
- **WHO – 2000’s**
  - B ALL
  - For 2008, B lymphoblastic stratified into cytogenetic subgroups (similar to the AML classification)
- **T ALL**
WHO Classification

- Based on a number of characteristics and biologic parameters
- Better correlates with disease biology, pathogenesis, prognosis
- Specific clinicopathologic syndromes when possible
- Not as easy to say as the FAB
What We Need to Fully Diagnose ALL/LBL by the WHO

- Integration of multiple modalities necessary to make a complete diagnosis:
  - **Morphology** – PB, BM, LN (or other tissue)
  - **Immunophenotype** – flow cytometry (or immunohistochemistry)
  - **Cytogenetics/molecular testing**
    - Important for sub classification, treatment, prognosis
  - **Clinical history**
  - (Cytochemistry) – largely replaced by flow cytometry, may give additional information in difficult cases with “mixed messages” (greater sensitivity for MPO expression)
B-ALL/T-ALL – Smear Morphology

- Blasts vary from small with scant cytoplasm, condensed chromatin, and indistinct nucleoli to larger cells with moderate amounts of light blue-gray cytoplasm, occasionally vacuolated, dispersed nuclear chromatin, and multiple variably prominent nucleoli.

- Some cases may have cytoplasmic blebs or uropods (hand mirror cells)
  - No particular diagnostic or prognostic significance.

- Some cases may have prominent azurophilic granules
  - Can be seen with t(9;22), but not specific for any type.
  - MPO negative.
  - Some may stain with acid phosphatase or acid esterases.
Lymphoblasts
ALL/LBL – Morphology on Tissue Sections

- Blasts monotonous with round to oval nuclei, may be indented or convoluted
- Nucleoli indistinct or small
- Finely dispersed chromatin
- Variable number of mitotic figures
- Diffuse pattern, may have a “starry sky” with lots of tingible body macrophages
B ALL - Cytochemistry

- Negative for myeloperoxidase & Sudan black-B
- PAS – may show blocky cytoplasmic positivity

Largely supplanted by immunophenotyping by flow cytometry (and/or immunohistochemistry)
Let’s Talk Immunophenotype, or Why Many Pathologists Hate Hemepath:

All the #@*!^ markers to remember!
Immunophenotypic Analysis in Leukemias

- Basic approach
  - Normal? or abnormal?
  - Myeloid? or lymphoid?
  - Mature? Or Immature?
- There are very few markers that are lineage specific
  - Promiscuous expression of markers is VERY common
  - Putting everything together & the level of expression of the markers can be very helpful
    - CD7 expression is usually much brighter in T-ALL than in AML, although it can be expressed in both
- Certain patterns can be predictive of the cytogenetics or the leukemic subtype
  - But not all cases follow the “rules”
### Criteria For Lineage Assignment

**WHO 2008 Diagnostic Criteria for Mixed Phenotype Acute Leukemias**

<table>
<thead>
<tr>
<th>Myeloid</th>
<th>Monocytic</th>
<th>B</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO positive</td>
<td>NSE, CD11c, CD14, CD64,</td>
<td>Strong CD19 plus at least 1 strong:</td>
<td>Cytoplasmic or surface CD3</td>
</tr>
<tr>
<td></td>
<td>lysozyme</td>
<td>CD79a, CD22, CD10</td>
<td></td>
</tr>
<tr>
<td>That’s all you</td>
<td>Need at least 2</td>
<td>Weak CD19 plus at least 2 strong:</td>
<td>cytoCD3 better by flow than IHC</td>
</tr>
<tr>
<td>need (flow,</td>
<td></td>
<td>CD79a, CD22, CD10</td>
<td>(IHC may pick up CD3ζ – not as</td>
</tr>
<tr>
<td>cytochemistry,</td>
<td></td>
<td></td>
<td>specific)</td>
</tr>
<tr>
<td>IHC count)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MPO = myeloperoxidase, NSE = non-specific esterase
### B cell Maturation

**Blasts – Bone marrow**

**Mature cells – Lymph node**

<table>
<thead>
<tr>
<th>Immature</th>
<th>Immature</th>
<th>Immature</th>
<th>Naive/Mantle</th>
<th>Follicle Center</th>
<th>Marginal Zone</th>
<th>Plasma cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>TdT</td>
<td>CD34</td>
<td>CD10</td>
<td>CD38</td>
<td>CD19</td>
<td>CD20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD22</td>
<td>HLA-DR</td>
<td>IgD</td>
<td>IgM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kappa</td>
<td>Lambda</td>
</tr>
</tbody>
</table>

*Henry’s Clinical Diagnosis & Management by Laboratory Methods, 21st ed., p.603*
B-cell development

Pro B-cell
- CD34
- TdT
- CD22
- CD19
- cCD79a
- CD10 (br)

Pre-pre B-cell
- CD34
- TdT
- CD22
- CD19
- cCD79a

Pre B-cell
- TdT
- CD22
- CD19
- CD10 (dim)
- CD20
- cCD79a
- Cyt-μ

Immature B-cell
- CD22
- CD19
- CD10 (dim)
- CD20
- cCD79a
- Cyt-μ
- sIgM

Resting mature B-cell
- B-cell
- CD22
- CD19
- CD20
- cCD79a
- sIgM/IgD

Dim/negative

CD45 increases intensity with maturation

Bright
Immature Markers

- Non lineage specific (any blasts may or may not express) – CD34, TdT
- CD45 has decreased expression, compared to lymphocytes
  - Some cases may be CD45 negative (more common in ALL than AML)
- Baby T cells – CD1a
- Baby myeloid cells – CD117
B cell Markers

- Immature or mature B cells
  - CD19, CD22, CD79a, PAX-5
  - CD10
    - Non-lineage specific as some T cells and mature granulocytes also express

- Mature B cells
  - Surface light chain
  - CD20
  - There are always exceptions, but in general this is true
B cell markers - development

- Early precursors have CD34 & TdT, decreased CD45, bright CD10, bright CD38, weaker CD22, absent CD20
- As mature, lose CD34 & TdT, increase CD45 and CD22, lose CD10, decrease CD38 and pick up CD20
- ALL will fall outside these normal maturation patterns
  - Not a problem at diagnosis with lots of blasts, but very important for MRD assessment
Hematogones: Normal maturation patterns for baby B cells

Earliest precursors

Intermediate & late

Mature B cells

ALL falls outside of these patterns
Hematogones

Normal maturation patterns for baby B cells

ALL falls outside of these patterns
T cell Maturation

Bone Marrow

Thymus

Lymph Node
Peripheral Bld

Henry’s Clinical Diagnosis & Management by Laboratory Methods, 21st ed., p.604
T cell Development

**Bone Marrow**
- Prothymocyte
  - CD34
  - TdT
  - HLA-DR
  - CD2
  - CD7
  - cCD3

**Thymus**
- Immature Thymocyte
  - TdT
  - CD2
  - CD7
  - cCD3
  - CD25
  - TCR-r
- Common thymocyte
  - CD2
  - CD7
  - Pre-TCR
  - CD3 dim
  - CD5
  - CD1a
  - CD4 & CD8
- Mature thymocyte/Naïve T cell
  - CD2
  - CD7
  - TCR
  - CD3
  - CD5
  - CD4 or CD8
- Activated T cell
  - CD2
  - CD7
  - TCR
  - CD3
  - CD5
  - CD4 or CD8
  - CD25
  - HLA-DR

**CD45 increases intensity with maturation**
- Dim/negative
- Bright
T cell Markers

- Immature or Mature T cells
  - CD7, CD2, CD5, CD3 (cytoplasmic)
  - CD4 & CD8 both negative = immature
  - CD4 & CD8 both positive = immature
  - CD1a = immature

- Mature T cells
  - CD3 (surface)
  - Surface T cell receptor
  - CD4 or CD8
T cell development

- Earliest T cell precursors have cCD3+, TdT+, CD34+, leave bone marrow quickly and complete development in the thymus.

- Baby T cells should never be present in the bone marrow, so MRD for T-ALL is sort of easier.
  - cCD3+ and TdT+ = ALL in the marrow
  - But T-ALL tends to “mature” with therapy, so may hide with the normal T cells.
Myeloid & Monocytic Markers

- There is a lot of overlap between them
- Some AMLs may express T cell markers CD2, CD4, and/or CD7, but level of expression often lower than for normal T cells
  - Can lead to lineage confusion
- Myeloid
  - CD13, CD33, myeloperoxidase (the only really lineage specific one) come up early
  - CD15, CD16, CD11b, CD11c all come up later, at different times
Let’s Focus on B-ALL
ALL in WHO 2008: B Precursor Neoplasms

- B lymphoblastic leukemia/lymphoma, NOS
- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
  - With t(9;22)(q34;q11.2); **BCR-ABL1**
  - With t(v;11q23); **MLL** rearranged
  - With t(12;21)(p13;q22); **TEL-AML1 (ETV6-RUNX1)**
  - With hyperdiploidy
  - With hypodiploidy
  - With t(5;14)(q31;q32); **IL3-IGH**
  - With t(1;19)(q23;p13.3) **E2A-PBX1 (TCF3-PBX1)**
B ALL – Immunophenotype

- B-cell markers: CD19, CD79a, CD22, PAX-5
  - CD20 is a more mature B-cell marker, variable positivity in ALL
  - Surface Ig (another mature marker) typically absent
- Immature markers
  - CD34, TdT
- Non-lineage specific markers
  - CD10 (usu very bright), HLA-DR
- CD45 dim to absent, but variable patterns of expression are seen
B-ALL Immunophenotype – Aberrant Antigen Expression

- Myeloid markers (common)
  - CD13, CD33, CD15
  - Seen associated with t(9;22), t(4;11), t(12;21)
- T-cell and NK markers (uncommon)
  - CD2, CD7, CD56, CD57
B ALL – Immunophenotype & Underlying Genetics

- May correlate with underlying genetics
- \( t(9;22) \)
  - Bright CD10, dim/absent CD38, heterogenous expression of CD33+, CD13, **CD25**, CD66
- \( t(4;11) \) – “Pro-B” phenotype
  - CD10 & CD24 negative, positive for CD15 & CD65, NG2+
- \( t(12;21) \)
  - CD19+, CD10+, CD34+, TdT+
  - Commonly **CD13**+
  - Negative CD20 plus negative or partial positive CD9 most predictive
- Cytoplasmic \( \mu \) heavy chain expression common with \( t(1;19) \), but not totally specific
B ALL – The Classic Genetic Subtypes

- Important & predictive for prognosis, treatment stratification

- Recognized subtypes:
  - t(9;22) \( BCR-ABL1 \) – 3-4%
  - 11q23 \( MLL \) rearrangements – 2-3% (higher in infants)
  - t(12;21) \( TEL-AML1 \) (\( ETV6-RUNX1 \)) – cryptic, require FISH or molecular testing to identify – 16-29%
  - t(1:19) \( PBX-E2A \) (\( PBX-TCF3 \)) – 6%
  - Hyperdiploid > 50 (“triple trisomies” 4, 10, 17) – 20-25%
  - Hypodiploid – 5%
  - t(5;14); \( IL3-IgH \) - <1%, associated with eosinophilia
The Current Genetic Landscape for B-ALL

Harrison, CJ ASH Education Book 2013 Fig 1 p. 119
The Current Genetic Landscape for B-ALL

Harrison, CJ ASH Education Book 2013 Fig 1 p. 119
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Frequency (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploidy (&gt;50 chromosomes)</td>
<td>20–30</td>
<td>Excellent prognosis with antimetabolite-based therapy</td>
</tr>
<tr>
<td>Hypodiploidy (&lt;44 chromosomes)</td>
<td>1–2</td>
<td>Poor prognosis, high frequency of RAS pathway and IKAROS gene family mutations</td>
</tr>
<tr>
<td>t(12;21)(p13;q22) ETV6-RUNX1</td>
<td>15–25</td>
<td>Expression of myeloid antigens; excellent outcome</td>
</tr>
<tr>
<td>t(1;19)(q23;p13) TCF3-PBX1</td>
<td>2–6</td>
<td>Increased incidence in African Americans; generally excellent prognosis; association with CNS relapse</td>
</tr>
<tr>
<td>t(9;22)(q34;q11.2) BCR-ABL1</td>
<td>2–4</td>
<td>Historically dismal outcome, improved with addition of imatinib to intensive chemotherapy</td>
</tr>
<tr>
<td>PAX5 rearrangement</td>
<td>~2%</td>
<td>Multiple partners, commonly from dic(7;9), dic/t(9;12) and dic(9;20); outcome unknown</td>
</tr>
<tr>
<td>ABL1, PDGFRB, JAK2 rearrangements</td>
<td>2–5</td>
<td>Identified in BCR-ABL1–like ALL; multiple rearrangements encoding chimeric proteins, fusing 5′ partners with 3′ kinase domains; associated with IKZF1 alteration and very high leukocyte count; potentially amenable to tyrosine kinase inhibitor therapy</td>
</tr>
<tr>
<td>t(4;11)(q21;q23) MLL-AF4</td>
<td>1–2</td>
<td>Common in infant ALL (especially &lt;6 months of age); poor prognosis</td>
</tr>
<tr>
<td>MYC rearrangement [t(8;14)(q24;q32), t(2;8)(q12;q24)]</td>
<td>2</td>
<td>Favorable prognosis with short-term, high-dose chemotherapy</td>
</tr>
<tr>
<td>CRLF2 rearrangement (IGH@-CRLF2, PAR1 deletion, and P2RY8-CRLF2)</td>
<td>5–7</td>
<td>Extremely common in DS ALL (55%); association with IKZF1 deletion/mutation and JAK1/2 mutation; poor prognosis in non-DS ALL</td>
</tr>
<tr>
<td>ERG deletion</td>
<td>7</td>
<td>Subtype of B-ALL with a distinct gene expression profile; favorable outcome</td>
</tr>
</tbody>
</table>
The Current Genetic Landscape for B-ALL: Commonly Deleted Genes

- CDKN2A/B: 27%
- RB1: 7%
- PAX5: 19%
- IKZF1: 13%
- ETV6: 22%
- BTG1: 6%
- P2RY8-CRLF2: 4%
- EBF1: 2%
The Current Genetic Landscape for B-ALL: Commonly Mutated Genes

- **JAK1**: 2%
- **TBL1XR1**: 2%
- **CREBBP**: 2%
- **JAK2**: 9%
- **ETV6**: 4%
- **CDKN2A**: 1%
- **RB1**: 1%
- **TP53**: 4%
- **IKZF1**: 3%
- **PAX5**: 15%
- **NRAS**: 17%
- **KRAS**: 16%
- **PTPN11**: 5%
- **FLT3**: 7%
- **NF1**: 3%

Harrison, CJ ASH Education Book 2013 Fig 3 p. 120
<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Frequency</th>
<th>Pathophysiologic and clinical consequences of alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX5</td>
<td>Deletions, translocations, sequence mutations</td>
<td>One-third of B-progenitor ALL cases</td>
<td>Transcription factor required for B-lymphoid development; mutations impair DNA binding and transcriptional activation; cooperates in leukemogenesis, but no association with outcome</td>
</tr>
<tr>
<td>IKZF1</td>
<td>Focal deletions or sequence mutations</td>
<td>15% of all pediatric B-ALL cases, &gt; 70% of BCR-ABL1 lymphoid leukemia, and one-third of BCR-ABL1+ B-ALL</td>
<td>Transcription factor required for lymphoid development; deletions and mutations result in loss of function or dominant-negative isoforms; cooperates in pathogenesis of BCR-ABL1+ ALL; associated with poor prognosis in BCR-ABL1+ and BCR-ABL1- B-ALL</td>
</tr>
<tr>
<td>JAK1/2</td>
<td>Pseudokinase and kinase domain mutations</td>
<td>18%-35% DS-ALL and 10% high-risk BCR-ABL1-ALL; JAK1 mutations also identified in T-ALL</td>
<td>Results in JAK-STAT activation in model cell lines and primary leukemia cells; may be responsive to JAK inhibitors</td>
</tr>
<tr>
<td>CRLF2</td>
<td>Rearrangement as IGH@-CRLF2 or P2RY8-CRLF2 resulting in overexpression; F232C mutations</td>
<td>5%-16% pediatric and adult B-ALL and &gt; 50% DS-ALL</td>
<td>Associated with mutant JAK in up to 50% of cases; associated with IKZF1 alteration and poor outcome, particularly in non-DS-ALL</td>
</tr>
<tr>
<td>IL7R</td>
<td>Complex in-frame mutations in the transmembrane domain</td>
<td>Up to 7% of B- and T-ALL</td>
<td>Results in receptor dimerization and constitutive IL7R signaling and JAK-STAT activation; JAK inhibitors may also be useful</td>
</tr>
<tr>
<td>CREBBP</td>
<td>Focal deletion and sequence mutations</td>
<td>19% of relapsed ALL; commonly acquired at relapse</td>
<td>Mutations result in impaired histone acetylation and transcriptional regulation associated with glucocorticoid resistance</td>
</tr>
<tr>
<td>TP53</td>
<td>Deletions and sequence mutations</td>
<td>Up to 12% of B-ALL; commonly acquired at relapse</td>
<td>Loss of function or dominant negative; associated with poor outcome</td>
</tr>
<tr>
<td>Kinase rearrangements and mutations</td>
<td>Rearrangements of ABL1, PDGFRB, EPOR, JAK2, deletions of SH2B3</td>
<td>Present in half of BCR-ABL1-like ALL cases</td>
<td>Result in kinase signaling activation that is attenuated with TKIs</td>
</tr>
</tbody>
</table>

The table lists recently identified recurring genetic alterations in B-progenitor ALL that have key roles in leukemogenesis, risk stratification, and/or therapeutic targeting.
iAMP21

- Seen in 2% of B-ALL
- Region of amplification large but variable
  - At least 3 copies of \textit{RUNX1}
  - Often with deletion sub-telomeric region of 21
- Seen in older children and adolescents
- Associated with poor outcome
Event Free Survival – iAMP21

**UKALL2003**
5yrs EFS = 78%

**ALL97/99**
5yrs EFS = 29%

Treated as HR

Treated as SR

Harrison, CJ ASH Education Book 2013 Fig 2 p. 120
PAX-5

- B cell transcription factor, needed for B cell development
- Deletions, mutations, rearrangements in 1/3 of B-ALL
- Leads to haploinsufficiency
- No particular association with outcome
IKZF1 - Ikaros

- IKZF1 is required for lymphoid development
- Alterations present in 15% of B-ALL
- Seen in up to 1/3 of BCR-ABL1 negative HR B-ALL
- 80% of BCR-ABL1+ ALL
- Associated with adverse outcome, increased risk of treatment failure
CRLF2

- Cytokine receptor-like factor 2
  - Also known as thymic stromal-derived lymphopoietin (TSLP) receptor
  - With IL-7Rα, forms heterodimeric receptor for TSLP
  - Located at pseudoautosomal region at Xp22.3/Yp11.2
  - Rearranged or mutated in 5-16% of B-ALL
    - IgH-CRLF2, P2RY8-CRLF2
    - Results in surface overexpression of CRLF2, can be detected by flow cytometry
CRLF2 Rearrangements

- Present in 50% of Down syndrome (DS) related ALL
- Present in 50% of BCR-ABL1-like ALL
- Associated with activating mutations in *JAK1* and *JAK2*
  - Different *JAK2* mutation than seen in MPNs
- Outside of DS, associated with *IKZF1* alteration, *JAK* mutation, poor outcome
Figure 4. Simplified representation of signaling pathways associated with CRLF2 rearrangements and JAK mutations in which TSLP induces phosphorylation of STAT5, PI3K, and ERK. Potential therapeutic inhibitors and their targets are indicated.
BCR-ABL1 - like

- 15% of childhood ALL has a gene expression signature similar to BCR-ABL1 positive ALL
- **Very poor outcome**
- Often have deletion/mutation of *IKZF1*
- 50% have *CRLF2* rearrangements
- Studies show rearrangements, mutations, and CNAs activating kinase signaling
  - *PDGFRB, ABL1, JAK2, EPOR, SH2B3, IL7R*
  - Many result in downstream activation of JAK/STAT signaling pathways
  - Potential therapeutic targets
B ALL - Prognosis

- Risk assessment
  - Based on age, cytogenetics, WBC count, gender, response to initial therapy
  - >50% of kids will have good prognostic cytogenetics

- Good prognostic factors
  - Age 4-10, hyperdiploid chromosomes (triple trisomy), low or normal WBC at diagnosis

- Adverse prognostic factors
  - Very young age <1 year (associated with *MLL* rearrangements), t(9;22), t(4;11), near haploidy, positive MRD at end of induction (day +29), high WBC count at presentation, age >10 years
B ALL – Prognosis Based on Genetics (UK ALL97/99 trials)

- **Good risk**
  - High hyperdiploidy, ETV6-RUNX1 (TEL-AML1)

- **Intermediate risk**
  - Normal karyotype, all others

- **Poor risk**
  - t(9;22), iAMP21, MLL translocations, near haploidy, low hypodiploidy, t(17;19)(q23;p13), abnormal 17p, loss of 13q
Event Free Survival – ALL97/99

Harrison, CJ ASH Education Book 2013 Fig 2 p. 120
MRD: A Proven Powerful Prognostic Indicator in Leukemias

When do we know a “negative marrow” is really negative?
Leukemia Detection After Therapy

- Standard morphology – aspirate smears
- Flow cytometry
- Cytogenetics
  - Routine karyotyping
  - FISH
- Molecular techniques
  - Quantitative PCR or RT-PCR
MRD is Important

Early Responses to Chemotherapy of Normal and Malignant Hematologic Cells Are Prognostic in Children With Acute Lymphoblastic Leukemia

Stephen J. Laughton, Lesley J. Ashton, Edward Kwan, Murray D. Norris, Michelle Haber, and Glenn M. Marshall


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**Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood**


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**Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia**

Elaine Coustan-Smith, Jose Sancho, Michael L. Hancock, James M. Boyett, Frederick G. Behm, Susana C. Raimondi, John T. Sandlund, Gaston K. Rivera, Jeffrey E. Rubnitz, Raul C. Ribeiro, Ching-Hon Pui, and Dario Campana

*Blood*, 15 October 2000 • VOLUME 96, NUMBER 8
Issues with MRD Detection

- Different methods/targets used by different centers
  - Difficult to compare across studies
- Standardization & reproducibility
- Reference lab vs individual centers
- Viable vs dead/dying leukemia cells
- Comparison of different methods across patients
- What do we do with the results?
Characteristics of Ideal Assay for MRD

- Patient (or leukemia) specific
- High sensitivity (1 leukemic blast in 10,000 normal cells)
- Broad applicability to majority of patients
- Feasibility
- Intra- & inter-laboratory reproducibility
- Precise quantification of MRD levels

Szczepanski T. *Leukemia* 2007 21, 622-626
Routine Methods - Sensitivity

- Morphology: 5%
- Routine cytogenetics: 1-10%
- FISH: 1-5%

Not sensitive enough for MRD analysis
More Sensitive Methods

- Flow Cytometry
- Molecular methods – quantitative PCR
  - Fusion gene
  - TCR/IgH genes - clonality
Flow Cytometry

- Cocktail of fluorescently labeled antibodies (4+ colors)
  - Current COG assay is 6 colors

- What we’re looking at
  - Surface/cytoplasmic protein (antigen) expression
  - Specific immunophenotype for the patient

- Sensitivity: $10^{-3}$ to $10^{-4}$
  - Can get higher sensitivity with incr # colors (6+)

- Applicability: >95% of ALLs
Flow Cytometry: Advantages

- Applicable for most patients
  - Look for leukemia associated immunophenotypes (LAIP)
- Discern viable from nonviable cells
- Relatively cheap
- Rapid turn around time (1-2 days)
Flow Cytometry: Disadvantages

- Limited sensitivity compared to molecular techniques
- Immunophenotype may change therapy or at relapse
  - Drop or pick up new markers
  - Levels of antigen expression may change
  - Need preferably two aberrant phenotypes per aberrant population
- If the pattern overlaps too much with hematogones, you may miss it
PCR Analysis of Chromosome Aberrations (Fusion Genes)

- Quantitative RT-PCR for specific chromosomal abnormality – usually fusion gene (ex. \textit{TEL-AML1} or \textit{BCR-ABL})
  - Usually dealing with mRNA
- Sensitivity: $10^{-4}$ to $10^{-6}$
- Applicability: 40-45\% of Precursor B ALL’s, 15-35\% of T-ALL’s
PCR - Advantages

- Relatively easy & cheap
- High sensitivity
- Leukemia specific
- Stable target during disease course
- Rapid turn around time (2-3 days)
- Suitable for following patient groups
PCR - Disadvantages

- Useful only in minority of patients
- Doesn’t discern viable cells
- Not patient-specific
  - Be wary of contamination across samples or false positives
- Differences in fusion transcript expression levels between patients
- Stability of RNA
Gene Rearrangements

- Quantitative PCR for TCR & Ig junctional region rearrangements
  - TCRg, TCRd, IgH, IgK
  - Look for clonal signal against polyclonal background
  - Patient’s clone is sequenced & primers made to detect patient’s particular sequence

- DNA
- Sensitivity: $10^{-4}$ to $10^{-5}$
- Applicability: most ALL’s
- The technique used in Europe
Gene Rearrangements: Advantages

- Applicable for virtually all patients
- High sensitivity
- Patient specific
- Relatively rapid turn around time in follow up (2-3 days)
Gene Rearrangements: Disadvantages

- Time consuming at diagnosis – identifying the junctional regions
- Relatively expensive
- Need for preferably 2 PCR targets per patient – beware clonal evolution & mutations of junctional regions
- At very low levels, may get nonspecific amplification of normal cells there – false positives
- Difficult to increase sensitivity against normal polyclonal background
MRD Summary

- Various methods available with high sensitivity
- Important to help further risk stratify patients
  - We don’t know yet if treating based on MRD will make a difference
- No gold standard
  - COG uses flow in reference centers (Baltimore, Seattle)
    - Currently changing – working towards standardized testing at local centers
  - AIEOP, BFM use PCR for gene rearrangements, standardized & performed in multiple centers
- Potentially not as helpful for CNS or other extramedullary relapse
- Studies are on going . . .
COG Studies: Flow MRD is a Powerful Prognosticator

End Induction Bone Marrow MRD

Borowitz et al. Blood 2008 111(12) Fig 1 p. 5479.
End Induction BM

Positive = MRD >0.01%

A: TEL-AML1

\[ P < .0001 \]

- **Negative (\( \leq 0.01\%) (n = 369)\)**
- **Positive (\( > 0.01\%) (n = 37)\)**

95\( \pm \)2\%

56\( \pm \)12\%
Day 8 PB MRD

Event-free survival probability

- MRD Negative (≤ 0.01%) (n = 597)
- 0.01% < MRD ≤ 0.1% (n = 339)
- 0.1% < MRD ≤ 1.0% (n = 497)
- 1.0% < MRD ≤ 10.0% (n = 372)
- MRD > 10% (n = 115)

P < .0001

Table 1. Risk Groups for B-Precursor Acute Lymphoblastic Leukemia\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Low Risk</th>
<th>Average Risk</th>
<th>High Risk</th>
<th>Very High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI Risk (Age/WBC)</td>
<td>SR</td>
<td>SR</td>
<td>SR</td>
<td>SR</td>
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<tr>
<td></td>
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<tr>
<td>Favorable Genetics</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Unfavorable Characteristics</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 8 PB MRD</td>
<td>&lt;0.01%</td>
<td>≥0.01%</td>
<td>&lt;1%</td>
<td>Any Level</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Day 29 Marrow MRD</td>
<td>&lt;0.01%</td>
<td>&lt;0.01%</td>
<td>&lt;0.01%</td>
<td>≥0.01%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% of Patients (Estimated)</td>
<td>15%</td>
<td>36%</td>
<td>25%</td>
<td>24%</td>
</tr>
<tr>
<td>Anticipated 5-year EFS</td>
<td>&gt;95%</td>
<td>90%–95%</td>
<td>88%–90%</td>
<td>&lt;80%</td>
</tr>
</tbody>
</table>

EFS = event-free survival; HR = age and WBC count risk group is high risk; MRD = minimal residual disease; NCI = National Cancer Institute; PB = peripheral blood; SR = age/WBC count risk group is standard risk; WBC = white blood cell.

\(^a\)From the Children's Oncology Group Classification of Newly Diagnosed ALL protocol.
ALL in WHO 2008:
T Precursor Neoplasms

- T lymphoblastic leukemia/lymphoma

No specific cytogenetic abnormalities are considered to define separate entities, so all encompassed in one group - no splitting here!
T ALL - Immunophenotype

- T-cell markers: cytoplasmic CD3, CD2, **CD7** (usually bright), CD5
  - Surface CD3 often negative (more mature T cell marker)
  - CD1a positive – immature T cells
  - CD4+/CD8+, CD4-/CD8- most common, fewer are single positive CD4 or CD8

- Immature markers
  - TdT, CD34

- HLA-DR typically negative

- May express CD10

Note: No clear correlation with cytogenetics, unlike B ALL
T-ALL Immunophenotype

- Myeloid antigens may be co-expressed
  - CD13, CD33, CD117, CD15
- NK cell/ cytotoxic markers may be expressed
  - CD56, CD57, CD16
If very bright CD7, most likely to be T ALL even if myeloid markers are expressed.
Cytogenetic Subtypes of ALL

Mullighan, CG J Clin Invest 2012 122(10) pp. 3407-3415, Fig 1
Genetics of T-ALL

- 50% have abnormal karyotype
- Rearrangements commonly involve T cell receptors
  - TCRA and TCRD at 14q11, TCRB at 7q35
  - Result in dysregulated expression of partner genes
- Cryptic rearrangements may form abnormal fusion genes
Cytogenetics of T-ALL

- t(7;10)(q34;q24) & t(10;14)(q24;q11) \(HOX11\)
  - 7% of pediatric T-ALL
- t(1;14)(p32;q11) & t(1;7)(p32;q35) \(TAL1\)
  - <5% of T-ALL
- Cryptic interstitial deletion of 1p32 \(SIL-TAL1\)
  - 30% of pediatric T-ALL
- t(10;11)(p13;q14) \(CALM-AF10\) in 10%
- \(NUP214-ABL1\) in 6%
- Deletions of 9p21 \(CDKN2A\) gene common
Genetics of T-ALL

- Activating mutations of NOTCH1 in >50%
  - May have better relapse free survival
- Gene expression profiling has identified different signatures
  - LYL1, HOX11, TAL1
  - Correlate with stages of T cell development
  - HOX11 (corr with cortical thymocytes) – better prognosis
- ETP-ALL also recently described, originally identified by GEP studies
T ALL - Prognosis

- Treated as high risk disease in kids
  - Survival on current regimens better than in past
- Increased risk for treatment failure, early relapse, isolated CNS relapse
- Poor prognosis if positive MRD at end of induction
  - Note: MRD is sort of easier to do – no confusion with hematogones & you should not have CD3+ TdT positive cells in the bone marrow
  - But markers often mature with treatment, making separation from normal T cells sometimes challenging
ETP-ALL:
Baby T-cells gone VERY VERY bad
Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia

Elaine Coustan-Smith, Charles G Mullighan, Mihaela Onciu, Frederick G Behm, Susana C Raimondi, Deqing Pei, Cheng Cheng, Xiaoping Su, Jeffrey E Rubnitz, Giuseppe Basso, Andrea Biondi, Ching-Hon Pui, James R Downing, Dario Campana

Lancet Oncol 2009; 10: 147–56

Do a subset of T-ALL derive from ETPs?
Having myeloid/stem cell potential – do they do worse?
Yes!!!
Early T-precursors

- Recently recognized
- Subset of thymocytes derive from early recent immigrant from the bone marrow
- Retain multilineage potential for T and myeloid differentiation
- Stem cell-like qualities
- Unique signature with GEP & unique immunophenotype
- This phenotype seen in up to 13% of T-ALL
Stages in early T cell development

Fig 1. Rothenberg, Moore, and Yui. Nature Reviews Immunology. Jan 2008 8; 9-21
T cell Development

Bone Marrow

Prothymocyte
- CD34
- TdT
- HLA-DR
- CD2
- CD7
- cCD3

Immature Thymocyte
- TdT
- CD2
- CD7
- cCD3
- CD25
- TCR-r

Common thymocyte
- CD2
- CD7
- Pre-TCR
- CD3 dim
- CD5
- CD1a
- CD4 & CD8

Mature thymocyte/Naïve T cell
- CD2
- CD7
- TCR
- CD3
- CD5
- CD4 or CD8

Activated T cell
- CD2
- CD7
- TCR
- CD3
- CD5
- CD4 or CD8
- CD25
- HLA-DR

CD45 increases intensity with maturation
- Dim/negative
- Bright
Fig 2A

St. Jude’s cohort – 55 pts
ETP-ALL Immunophenotype

- Negative for CD1a, CD8
- Weak to negative expression of CD5 (<75% of lymphoblasts positive)
- Expression of at least one myeloid or stem cell marker in at least 25% of lymphoblasts
  - CD11b, CD13, CD33, CD34, CD65, CD117, or HLA-DR
ETP Immunophenotype
Figure 4: Prevalence of minimal residual disease (MRD) during the early phases of therapy for patients with early T-cell precursor (ETP) or typical T-lymphoblastic leukaemia (T-ALL). MRD levels were measured by flow cytometry (A) or by PCR amplification of antigen-receptor genes (B). Horizontal bars indicate median values, if above 0.01%.
Event Free Survival

St. Jude’s cohort

AIEOP cohort

Fig 5B,E
Cumulative incidence of remission failure or hematological relapse

St. Jude’s cohort

AIEOP cohort

Fig 5 C,F
Table 2: Univariate and multivariate analysis of event-free survival according to the diagnosis of ETP-ALL and selected variables in patients in the St Jude cohort

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
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<th>Mutivariate</th>
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<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>p value</td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>p value</td>
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<td>ETP-ALL</td>
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<tr>
<td>Yes vs no†</td>
<td>4.58</td>
<td>2.25-9.33</td>
<td>&lt;0.0001</td>
<td>10.65</td>
<td>3.73-30.42</td>
<td>&lt;0.0001</td>
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<td>Sex</td>
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<tr>
<td>Male vs female</td>
<td>1.09</td>
<td>0.52-2.30</td>
<td>0.82</td>
<td>1.24</td>
<td>0.57-2.69</td>
<td>0.59</td>
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<td>Ethnic origin‡</td>
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<tr>
<td>White vs other</td>
<td>0.76</td>
<td>0.39-1.46</td>
<td>0.40</td>
<td>0.95</td>
<td>0.44-2.04</td>
<td>0.89</td>
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<td>Age (years)§</td>
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<tr>
<td>&lt;1 vs 1-10</td>
<td>1.81</td>
<td>0.24-13.45</td>
<td>0.56</td>
<td>1.62</td>
<td>0.18-14.31</td>
<td>0.66</td>
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<tr>
<td>&gt;10 vs 1-10</td>
<td>0.81</td>
<td>0.42-1.56</td>
<td>0.53</td>
<td>0.42</td>
<td>0.18-1.00</td>
<td>0.05</td>
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<td>WBC (x10^9/L)§</td>
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<td>≥10-49 vs &lt;10</td>
<td>0.86</td>
<td>0.34-2.19</td>
<td>0.75</td>
<td>0.85</td>
<td>0.31-2.35</td>
<td>0.75</td>
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<tr>
<td>50-99 vs ≤10</td>
<td>0.20</td>
<td>0.04-0.94</td>
<td>0.04</td>
<td>0.14</td>
<td>0.03-0.68</td>
<td>0.02</td>
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<tr>
<td>≥100 vs ≤10</td>
<td>0.99</td>
<td>0.43-2.27</td>
<td>0.99</td>
<td>0.92</td>
<td>0.35-2.37</td>
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<td>CNS involvement</td>
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<tr>
<td>Yes vs no</td>
<td>1.84</td>
<td>0.97-3.51</td>
<td>0.06</td>
<td>2.38</td>
<td>1.06-5.33</td>
<td>0.04</td>
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<td>Mediastinal mass</td>
<td></td>
<td></td>
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<tr>
<td>Yes vs no</td>
<td>0.98</td>
<td>0.51-1.89</td>
<td>0.95</td>
<td>1.39</td>
<td>0.54-3.55</td>
<td>0.49</td>
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<tr>
<td>Treatment protocol</td>
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<tr>
<td>XIII and XIV vs XV¶</td>
<td>1.15</td>
<td>0.58-2.27</td>
<td>0.69</td>
<td>0.88</td>
<td>0.34-2.33</td>
<td>0.80</td>
</tr>
<tr>
<td>MRD on day 43</td>
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<td></td>
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</tr>
<tr>
<td>≥0.01% vs &lt;0.01%</td>
<td>2.30</td>
<td>1.12-4.72</td>
<td>0.02</td>
<td>1.30</td>
<td>0.56-3.03</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*By the Cox proportional-hazards model. †Based on the 17 patients with ETP-ALL versus the 114 with a typical immunophenotype (eight patients were excluded because of incomplete information on one or more variable). ‡As reported by parents (or patients, if older than 18 years of age). §Age and WBC were also analysed as continuous variables. They were not significant predictors and did not substantially change the results of the multivariate analysis when included as such in the model (data not shown). ¶Because of the small number of patients enrolled in Study XIV (see webappendix), this cohort was combined with patients in Study XIII.