Heterogeneity of Abnormal *RUNX1*
Leading to Clinicopathological Variations in Childhood B-Lymphoblastic Leukemia

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Background

- **RUNX1**
  - Runt-related transcription factor 1
  - Also known as:
    - Acute myeloid leukemia 1 protein (AML1)
    - Core-binding factor subunit alpha-2 (CBFA2)
- **RUNX1** gene – chromosome 21q22
- Function – participation in hematopoiesis
RUNX1 Function
Participation in Hematopoiesis

RUNX1 Abnormalities in Acute Leukemia

- **Translocations**
  - \textit{ETV6-RUNX1}/t(12;21)(p13;q22) \rightarrow \textit{childhood B-ALL (25%)} with good prognosis
  - \textit{RUNX1-RUNX1T1}/t(8;21)(q22;q22) \rightarrow \textit{AML with good prognosis}
  - \textit{RUNX1-MECOM}/t(3;21)(q26;q22) \rightarrow \textit{MDS & blastic phase of CML}

- **Amplifications** ($\geq 4$ \textit{RUNX1} copies on a single chromosome 21) \rightarrow \textit{childhood B-ALL (2%)} with unfavorable prognosis

- **Point Mutations** \rightarrow myeloid malignancies
Prognostic Significance of Chromosomal Abnormalities

UK ALL Trials
Study Objectives

Compare how abnormalities of *RUNX1* affect the clinicopathological expression in childhood B-ALL.
MATERIALS AND METHODS

- **Case Selection**
  - Newly diagnosed B-ALL with \textit{RUNX1} amplification or \textit{ETV6-RUNX1}
  - \(< 20\) years of age
  - 1999-2013
  - Children’s Hospital Colorado (CHC)

- **Clinical Information** – age, gender, WBC, CSF, relapse, mortality

- **Flow Cytometry** – immunophenotype (\(\geq 20\%\)) and S-phase (\(\geq 10\%\))

- **Cytogenetics**

- **FISH Analysis** - Vysis LSI \textit{ETV6(TEL)-RUNX1(AML1)} extra signal dual-color probe set (Abbott Molecular)
Results

- *RUNX1* amplification – 10 cases
- *ETV6-RUNX1* – 67 cases
Results

**RUNX1 amplification/iAMP**

46,XX,add(21)(q22)(iAMP21)[14]
<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>WBC</th>
<th>CSF</th>
<th>Immunophenotype</th>
<th>S-phase (%)</th>
<th>Karyotypes</th>
<th>FISH for RUNXI</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13y/M</td>
<td>3.7</td>
<td>+</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, sigM+, K+, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate</td>
<td>1.0</td>
<td>46,XY,inv dup(21)(q22.13q22.3)del(21)(q22.3)</td>
<td>RUNXI x 5-10</td>
<td>Moved to other state</td>
</tr>
<tr>
<td>2</td>
<td>15y/M</td>
<td>2.1</td>
<td>-</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, sigM+, K+, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate</td>
<td>23.4</td>
<td>46,XY,der(21)(r21)(p11.2q12)dup(21q)(8)(46,XY[3])</td>
<td>RUNXI x 4-8</td>
<td>CR &amp; alive for 7 yrs &amp; moved to other state</td>
</tr>
<tr>
<td>3</td>
<td>12y/M</td>
<td>14.3</td>
<td>-</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, sigM+, K+, λ-, CD2-, CD7+, CD13-, CD33-, CD45 dim to moderate</td>
<td>3.3</td>
<td>46,XY,del(9)(q22.3)(9:?:)(q34q34),del(21)(q10)hst(21)(q22)add(21)(q22)</td>
<td>RUNXI x 5-10</td>
<td>CR and alive for 7 years</td>
</tr>
<tr>
<td>4</td>
<td>1y/F</td>
<td>8.5</td>
<td>-</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, sigM+, K+, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate</td>
<td>7.23</td>
<td>46,XX,del(7)(q32),der(21)(r21)(q11.2q22.3)</td>
<td>RUNXI x 5-10</td>
<td>CR and alive for 6 years</td>
</tr>
<tr>
<td>5</td>
<td>5y/M</td>
<td>6.6</td>
<td>-</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, ccd79a+, sigM+, K-, λ-, CD2-, CD2-, CD7-, CD13-, CD33-, CD45 moderate</td>
<td>9.88</td>
<td>47,XY,del(16)(p12),add(21)(q22)(3)</td>
<td>RUNXI x 4-10</td>
<td>CR and alive for 5 years</td>
</tr>
<tr>
<td>6</td>
<td>13y/F</td>
<td>3.0</td>
<td>-</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, K+, λ-, CD3-, CD3-, CD53-, CD45 dim</td>
<td>NA</td>
<td>47,XX,+,add(21)(q22)(12)/46,XX[8]</td>
<td>RUNXI &gt;5</td>
<td>CR and alive for 3 years</td>
</tr>
<tr>
<td>7</td>
<td>7y/F</td>
<td>6.9</td>
<td>-</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, ccd79a+, sigM+, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 moderate</td>
<td>5.8</td>
<td>46-47,XX,+,X,add(3)(p24),add(9)(q22),add(12)(q13),del(13)(q12q22),-21,+1~5mar,inc[cp4]/46,XX[20]</td>
<td>RUNXI x 4-8</td>
<td>CR and alive for 3 years</td>
</tr>
<tr>
<td>8</td>
<td>6y/F</td>
<td>5.5</td>
<td>+</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, ccd79a+, sigM+, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 dim to dim</td>
<td>3.8</td>
<td>46,XX[20]</td>
<td>RUNXI x 6-8</td>
<td>CR and alive for 2 years</td>
</tr>
<tr>
<td>9</td>
<td>19y/F</td>
<td>5.3</td>
<td>+</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, ccd79a+, sigM+, K-, λ-, CD2-, CD7-, CD13-, CD33-, CD45 dim</td>
<td>3.78</td>
<td>46,XY,del(21)(q21q22)+amp(AML1x5-10)(14)/46,XY,del(7)(q11.2)[4]/46,XY[2]</td>
<td>RUNXI x 5-9</td>
<td>CR and alive for 2 years</td>
</tr>
<tr>
<td>10</td>
<td>2y/F</td>
<td>10.6</td>
<td>+</td>
<td>CD34+, HLA-DR+, TdT wk+, CD10+, CD19+, CD20+, CD22+, ccd79a+, sigM+, K-, λ-, CD2-, CD7+, CD13-, CD33-, CD45 moderate</td>
<td>6.4</td>
<td>46,XX,add(21)(q22)(gAMP21)(14)/46,XX[6]</td>
<td>RUNXI x 4-7</td>
<td>CR and alive for 6 months</td>
</tr>
</tbody>
</table>
Aberrant Expression of CD7 Frequently Seen in B-ALL with \textit{RUNX1} Amplification Than B-ALL with \textit{ETV6-RUNX1}
### Table 2  Clinicopathologic Variations in Patients with Abnormal RUNX1

<table>
<thead>
<tr>
<th></th>
<th>RUNX1 amplification (group 1)</th>
<th>ETV6-RUNX1 with RUNX1 gain (group 2)</th>
<th>ETV6-RUNX1 without RUNX1 gain (group 3)</th>
<th>( P ) (G1 vs G2)</th>
<th>( P ) (G1 vs G3)</th>
<th>( P ) (G2 vs G3)</th>
<th>( P ) (G1 vs G2 &amp; G3)</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Cases</td>
<td>10</td>
<td>34</td>
<td>33</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.0051</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>10.1 (2 - 19)</td>
<td>5.1 (1 - 14)</td>
<td>3.5 (1 - 8)</td>
<td>0.7161</td>
<td>0.4809</td>
<td>1.0000</td>
<td>0.4990</td>
</tr>
<tr>
<td>(range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M:F</td>
<td>5:5</td>
<td>21:13</td>
<td>21:12</td>
<td>0.5592</td>
<td>0.5579</td>
<td>1.0000</td>
<td>0.5867</td>
</tr>
<tr>
<td>WBC ≥50,000/mm³</td>
<td>0/10 (0%)</td>
<td>4/34 (12%)</td>
<td>4/33 (12%)</td>
<td>0.6707</td>
<td>0.0733</td>
<td>0.1497</td>
<td>0.1835</td>
</tr>
<tr>
<td>CSF+</td>
<td>3/10 (30%)</td>
<td>7/34 (21%)</td>
<td>2/33 (6%)</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>Phenotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2+</td>
<td>0/9 (0%)</td>
<td>0/30 (0%)</td>
<td>0/33 (0%)</td>
<td>0.0015</td>
<td>0.0011</td>
<td>1.0000</td>
<td>0.0001</td>
</tr>
<tr>
<td>CD7+</td>
<td>4/9 (44%)</td>
<td>0/30 (0%)</td>
<td>0/33 (0%)</td>
<td>0.0212</td>
<td>0.1646</td>
<td>0.2972</td>
<td>0.0536</td>
</tr>
<tr>
<td>CD13+</td>
<td>0/10 (0%)</td>
<td>13/34 (38%)</td>
<td>8/32 (25%)</td>
<td>1.0000</td>
<td>0.7004</td>
<td>0.7785</td>
<td>1.0000</td>
</tr>
<tr>
<td>CD33+</td>
<td>2/10 (20%)</td>
<td>8/34 (24%)</td>
<td>9/31 (29%)</td>
<td>1.0000</td>
<td>0.5628</td>
<td>0.1953</td>
<td>1.0000</td>
</tr>
<tr>
<td>S-phase ≥10%</td>
<td>0/8 (0%)</td>
<td>1/31 (3%)</td>
<td>5/31 (16%)</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>Outcome</td>
<td>Relapse 1/10 (10%)</td>
<td>1/33 (3%)</td>
<td>4/31 (13%)</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>Mortality 0/8 (0%)</td>
<td>2/33 (6%)</td>
<td>1/31 (3%)</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

*RUNX1 amplification Group 1  
ETV6-RUNX1 with RUNX1 gain Group 2  
ETV6-RUNX1 without RUNX1 gain Group 3*
### Table 3: Aberrant Expression Myeloid-Associated Antigens in Subgroups of Patients with ETV6-RUNX1 Translocation

<table>
<thead>
<tr>
<th></th>
<th>ETV6-RUNX1 with RUNX1 gain (group 2)</th>
<th>ETV6-RUNX1 without RUNX1 gain (group 3)</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Double ETV6-RUNX1 fusions (group 2A)</td>
<td>Single ETV6-RUNX1 with wild type RUNX1 gain (group 2B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of Cases</td>
<td>13</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD13+</td>
<td>10/13 (77%)</td>
<td>3/21 (14%)</td>
<td>8/32 (25%)</td>
<td>0.0006</td>
<td>0.0022</td>
</tr>
<tr>
<td>CD13-</td>
<td>3/13 (23%)</td>
<td>18/21 (86%)</td>
<td>24/32 (75%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD33+</td>
<td>5/13 (38%)</td>
<td>3/21 (14%)</td>
<td>9/31 (29%)</td>
<td>0.2106</td>
<td>0.7241</td>
</tr>
<tr>
<td>CD33-</td>
<td>8/13 (62%)</td>
<td>18/21 (86%)</td>
<td>22/31 (71%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Group 2A**
- **ETV6-RUNX1** with gain of fusion

**Group 2B**
- **ETV6-RUNX1** with gain of wild type (wt) RUNX1

**Group 3**
- **ETV6-RUNX1** “Classic” t(12;21)
Aberrant Expression of Myeloid Antigens Is More Common in Double \textit{ETV6-RUNX1} Fusion Group Than a Single \textit{ETV6-RUNX1} with a Wild Type \textit{RUNX1} Gain Group
Result Summary

- **Mean age**
  - amplification group (10.1 y) older than translocation group (5.1 y)

- **Genders**
  - equal distribution in amplification group (M:F = 5:5)
  - male predominant in translocation group (M:F = 21:13)

- **Hyperleukocytosis**
  - translocation group (12%) > amplification group (0%)

- **CSF+**
  - amplification group (30%) > translocation group (13%)

- **Phenotype**
  - amplification group – **CD7**
  - translocation group – **CD13** and CD33
    - double translocations > single translocation with **RUNX1** gain

- **Outcomes**
  - amplification group with high risk treatment = translocation group
Conclusions

- Patients with RUNX1 amplification are older than patients with ETV6-RUNX1 suggesting that the factors driving amplification of RUNX1 may require longer time to develop or operate than those driving translocation of RUNX1.

- B-ALLs with RUNX1 amplification more frequently show aberrant expression of CD7, suggesting amplification of RUNX1 may prevent silencing of T-cell phenotype in B-lymphoblasts.

- B-ALLs with ETV6-RUNX1 carry aberrant myeloid markers more often than those with RUNX1 amplification suggesting that RUNX1 at 21q22 likely is a myeloid associated breakpoint as seen in AML with t(8;21)(q22;q22)/RUNX1-RUNX1T1.

- Increased number of ETV6-RUNX1 translocation, rather than gain of wild type RUNX1 promotes more frequent expression of myeloid-associated antigens in B-ALL.

- More frequent CNS involvement may be partially responsible for more aggressive clinical behavior in patients with RUNX1 amplification, although the differences are not statistically significant.

- Similar clinical outcome between RUNX1 amplification and ETV6-RUNX1 groups is attributed to different risk stratification treatments.
Contributors

- Virginia Knez, MD: University of Colorado Hospital
- Billie Carstens: Colorado Cytogenetic Laboratory
- Karen Swisshelm, PhD: Colorado Cytogenetic Laboratory
- Amy McGranahan: Children’s Hospital Colorado