Genetic alterations in high risk ALL
Insights into biology and therapeutic targets

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Pathology
St Jude Children’s Research Hospital
Memphis
Cytogenetics of pediatric ALL

B-lineage

Hypodiploidy
<45 chromosomes
1%

Hyperdiploidy
>50 chromosomes
25%

Others
22%

BCR-ABL
\[ t(9;22) \]
3%

MLL rearrangements
8%

\[ MLL-ENL \]
0.3%

MYC
\[ t(8;14), t(2;8), t(8;22) \]
2%

E2A-PBX1
\[ t(1;19) \]
5%

TAL1
7%

LYL1
1.5%

Fusion Genes

TEL-AML1
\[ t(12;21) \]
22%

HOX11
0.7%

MLL
rearrangements
8%

HOX11L2
2.5%

T-lineage

Genetics of ALL

- Known chromosomal alterations do not account for all the genetic lesions in ALL
- Frequency of recurring karyotypic alterations declines with increasing age
- Genetic basis of treatment failure poorly understood
  - Many relapses occur in cases that lack very high risk alterations (MLL, BCR-ABL1, hypodiploidy)
- Leverage genome-wide profiling of ALL
  - Lesions contributing to pathogenesis
  - Lesions determining relapse
  - Identify novel therapeutic targets
Dissecting the genetic basis of acute leukemia

Chromosomal Aberrations
- Aneuploidy
- Rearrangements

DNA copy number abnormalities
- Amplifications/Deletions
- Copy-neutral LOH
- Inherited copy number variants

Sequence mutations
Sequence polymorphisms

Epigenetic changes
- CpG methylation
- Histone acetylation
Data Acquisition

Leukemic Blasts

Extract DNA

leukaemia and normal cells

Label and Hybridization

Laser Excitation

Signal Acquisition

Data Analysis

Hyperdiploid >50

BCR-ABL

TEL-AML1 MLL

E2A-PBX1

T-ALL

leukaemia and normal cells

Data Analysis
Genetic alterations in diagnosis ALL samples

![Genetic alterations in diagnosis ALL samples graph]
SNP array analysis of diagnosis ALL samples

- 242 B-progenitor and T-lineage ALL cases
- Lack of genomic instability - ~6 lesions per case
- >50 recurring regions of deletion/gain. Most focal (<1Mb)
- Significant variation in lesion type and frequency between ALL subtypes:
  - \textit{MLL}: <1 lesion/case
  - \textit{BCR-ABL1, ETV6-RUNX1}: >8 lesions/case
- Lesions target key cellular pathways:
  - Lymphoid development: \textit{PAX5, EBF1, IKTZ1}: over 60% of cases
Genes regulating B lymphoid development are mutated in ~65% B-progenitor ALL.

Block in maturation in B-ALL.
SNP array analysis of diagnosis ALL samples

- 242 B-progenitor and T-lineage ALL cases
- Lack of genomic instability - ~6 lesions per case
- >50 recurring regions of deletion/gain. Most focal (<1Mb)
- Significant variation in lesion type and frequency between ALL subtypes:
  - **MLL**: <1 lesion/case
  - **BCR-ABL1, ETV6-RUNX1**: >8 lesions/case
- Lesions target key cellular pathways
  - Lymphoid development: **PAX5, EBF1, IKZF1**
  - Tumor suppressors: **CDKN2A/B, RB1, ATM, NF1**
  - Lymphoid signaling pathways: **BTLA, CD200, CRLF2**
  - Oncogene amplification: **MYB** (T-ALL)
  - DNA structure: Histone genes
  - Apoptosis regulation: **BTG1**
  - Drug response: **NR3C1** (glucocorticoid receptor)
- Role in treatment response?
Children’s Oncology Group P9906 study

- Childhood B-progenitor ALL
- High risk based on age, sex, leukocyte count (median age 13.3)
- Augmented Berlin-Frankfurt-Münster regimen; 2000-2003
- $\text{BCR-ABL1}$ positive, hypodiploid, or induction failures excluded
- No trisomy 4/10 or $\text{ETV6-RUNX1}$ unless CNS/testicular disease
- 221 cases studied
- 170 cases lacked a sentinel chromosomal lesion
- Genomic analysis (NCI TARGET* project)
  - Affymetrix 500K SNP array: all cases
  - Affymetrix U133 Plus 2.0 gene expression profiling: 198 cases
  - Candidate gene resequencing: $\text{PAX5, IKZF1, EBF1}$

*Therapeutically Applicable Research to Generate Effective Treatments
http://target.cancer.gov

### Copy number alterations (CNA)

<table>
<thead>
<tr>
<th>Deletion</th>
<th>COG 9906 (N=221)</th>
<th>St Jude B-ALL (N=258)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CDKN2A</strong></td>
<td>101 (46)</td>
<td>87 (34)</td>
</tr>
<tr>
<td><strong>PAX5 CNA</strong></td>
<td>70 (32)</td>
<td>79 (31)</td>
</tr>
<tr>
<td><strong>PAX5 CNA or mutation</strong></td>
<td>81 (37)</td>
<td>83 (32)</td>
</tr>
<tr>
<td><strong>IKZF1</strong></td>
<td>63 (29)</td>
<td>48 (19)</td>
</tr>
<tr>
<td><strong>IKZF1 CNA or mutation</strong></td>
<td>67 (30)</td>
<td>48 (19)</td>
</tr>
<tr>
<td><strong>ETV6</strong></td>
<td>28 (13)</td>
<td>63 (24)</td>
</tr>
<tr>
<td><strong>RB1</strong></td>
<td>25 (11)</td>
<td>15 (6)</td>
</tr>
<tr>
<td><strong>BTG1</strong></td>
<td>23 (10)</td>
<td>18 (7)</td>
</tr>
<tr>
<td>13q14.2 (miRNA)</td>
<td>21 (10)</td>
<td>16 (6)</td>
</tr>
<tr>
<td><strong>C20orf94</strong></td>
<td>19 (9)</td>
<td>20 (8)</td>
</tr>
<tr>
<td><strong>EBF</strong></td>
<td>17 (8)</td>
<td>12 (5)</td>
</tr>
<tr>
<td><strong>IL3RA/CSF2RA</strong></td>
<td>15 (7)</td>
<td>18 (7)</td>
</tr>
<tr>
<td><strong>DMD</strong></td>
<td>15 (7)</td>
<td>11 (4)</td>
</tr>
<tr>
<td><strong>B cell development pathway</strong></td>
<td>147 (67)</td>
<td>137 (53)</td>
</tr>
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</table>
**IKZF1** (Ikaros) alterations in high risk ALL

<table>
<thead>
<tr>
<th>Deletion</th>
<th>COG 9906 (N=221)</th>
<th>St Jude B-ALL (N=258)</th>
</tr>
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<tbody>
<tr>
<td><em>CDKN2A</em></td>
<td>46%</td>
<td>34%</td>
</tr>
<tr>
<td><em>PAX5</em> CNA or mutation</td>
<td>37%</td>
<td>32%</td>
</tr>
<tr>
<td><em>IKZF1</em> CNA or mutation</td>
<td>30%</td>
<td>19%</td>
</tr>
<tr>
<td>B cell development pathway</td>
<td>67%</td>
<td>53%</td>
</tr>
</tbody>
</table>

**IKZF1 deletions**

**IKZF1 mutations**
Predicting outcome using genome-wide copy number data

Development of Survival Predictor

Training Set
P9906
N=221

ALL

Test Set
St Jude
N=258

Validation of Survival Predictor

<table>
<thead>
<tr>
<th></th>
<th>P9906 (N)</th>
<th>St Jude (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCF3-PBX1</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>ETV6-RUNX1</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>High hyperdiploid</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>MLL-rearranged</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>BCR-ABL1</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Other</td>
<td>170</td>
<td>92</td>
</tr>
</tbody>
</table>
**IKZF1 alteration and poor outcome in ALL**

**Multivariable analysis: relapse**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard Ratio (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>Age</td>
<td>0.79 (0.4-1.6)</td>
<td>0.46</td>
</tr>
<tr>
<td>Subtype</td>
<td>1.10 (0.4-3.2)</td>
<td>0.86</td>
</tr>
<tr>
<td>WBC</td>
<td>1.21 (0.7-2.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>D29 minimal residual disease</td>
<td>2.55 (1.3-4.9)</td>
<td>0.0044</td>
</tr>
<tr>
<td>IKZF1 alteration</td>
<td>2.40 (1.38-4.2)</td>
<td>0.0021</td>
</tr>
</tbody>
</table>
IKZF1 (Ikaros) is deleted in 83.7% BCR-ABL1 ALL

<table>
<thead>
<tr>
<th>ALL subtype (N)</th>
<th>IKZF1 (Ikaros) (%)</th>
<th>CDKN2A (%)</th>
<th>PAX5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL1 de novo ALL (43)</td>
<td>84</td>
<td>53</td>
<td>51</td>
</tr>
<tr>
<td>- childhood (21)</td>
<td>76</td>
<td>62</td>
<td>57</td>
</tr>
<tr>
<td>- adult (22)</td>
<td>91</td>
<td>45</td>
<td>45</td>
</tr>
</tbody>
</table>

| Chronic myeloid leukemia         |                    |            |          |
|----------------------------------|                    |            |          |
| - chronic phase (N=60)           | 0                   | 0          | 0        |
| - lymphoid blast crisis (N=10)   | 70                  | 70         | 50       |
| - myeloid blast crisis (N=28)    | 18 (7p-/-7)         | 18         | 14       |

- Similar genetic abnormalities in *de novo* BCR-ABL1 ALL and lymphoid blast crisis CML
- Genomic lesions are critical determinants of the lineage of BCR-ABL1 leukemia

*Nature* 2008; 453: 110
IKAROS and ALL

- Zinc finger transcription factor required for lymphoid development
- Ikaros null mice lack all lymphoid lineages
- Aberrant IKZF1 isoforms in ALL
- Internal /IKZF1 deletions results in dominant negative isoforms
- Mice heterozygous for a dominant negative Ikaros mutation develop aggressive lymphoproliferative disease

\[ Ikzf1^{+/+} \text{ or } Ikzf1^{+/-} \text{ marrow} \]
\[ \downarrow \]
\[ MSCV-GFP-IRES-p185 BCR-ABL \]
\[ \downarrow \]
Transplant into irradiated recipients

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Racquel Collins-Underwood
A novel subtype of “BCR-ABL1-like” high risk ALL

- Gene set enrichment analysis shows similarity of signatures of Ph+ (IKZF1 deleted) and Ph- (IKZF1 deleted) ALL
- Implications of the “BCR-ABL1-like” subtype of ALL
  - IKZF1 alteration central to BCR-ABL1 positive and negative ALL
  - Unidentified kinase activating lesions?

also: den Boer et al Lancet Oncol 2009;10:125
JAK mutations in high-risk B-ALL

PNAS 2009; May 22 [Epub]
Modeling of JAK mutations

Brenda Schulman

Pseudokinase

Kinase
**JAK** mutations are transforming and sensitive to JAK inhibitors

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**Ba/F3 cells + Jak mutants**

![Graph showing cell growth over days for different Jak mutants](image1)

**Ba/F3 cells + Jak inhibitor**

![Graph showing normalized viability over Jak inhibitor concentration](image2)

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<table>
<thead>
<tr>
<th>JAK2</th>
<th>JAK1</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>617F</td>
<td>646F</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>682F</td>
<td>682F</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>683G</td>
<td>683G</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>683S</td>
<td>683S</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>873N</td>
<td>873N</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>933R</td>
<td>933R</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**JAK inhibitor I**

- pSTAT5
- STAT5
- PCNA
JAK mutations in ALL – other data

• JAK2 mutations in up to 28% Down syndrome ALL
  – Izraeli, Kearney, Rabin groups
• JAK1 mutations in (adult) T-lineage ALL (uncommon)
• No JAK mutations in non-DS B-progenitor ALL
• **BUT:**
  – Not comprehensive screening of all exons of all JAKs
  – Possible cohort dependence (Izraeli study enriched for low risk)
• Follow-up studies
  – SJ: JAK mutations in DS and non-DS ALL
  – Other COG HR and SR cohorts
  – AYA ALL
## JAK, IKZF1 and outcome

<table>
<thead>
<tr>
<th>Mutations</th>
<th>4 yr Relapse Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK + IKZF1</td>
<td>78% (p=0.0002)</td>
</tr>
<tr>
<td>IKZF1 only</td>
<td>54%</td>
</tr>
<tr>
<td>JAK only</td>
<td>33%</td>
</tr>
<tr>
<td>Neither</td>
<td>24%</td>
</tr>
</tbody>
</table>

![Graph showing relapse rates for different mutation combinations](image)
Unanswered questions

• What additional kinase alterations are present in “BCR-ABL1-like” high risk ALL
• Is JAK inhibition a useful therapeutic target, and if so, in which patients? Only those with JAK mutations, or all those with JAK-STAT activation?
• Why does *IKZF1* connote such poor prognosis?
• Which alterations should be explored as diagnostic markers?
• Validation in other cohorts/studies
• Biology
  – Leukemogenesis
  – Responsiveness to JAK inhibitors
Genomic analysis of AYA ALL

- Dearth of specific data
- Limited data in adult population (Paulsson PNAS 2008)
- P9906 cohort
  - 58 patients age 16-21, 50 lacked a sentinel chromosomal alteration

<table>
<thead>
<tr>
<th>Mutations</th>
<th>All pts</th>
<th>Age 16-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK + IKZF1</td>
<td>78%</td>
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<td>-</td>
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<tr>
<td>Neither</td>
<td>24%</td>
<td>18</td>
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</tbody>
</table>
Conclusions

• Genome-wide profiling has provided important insights into the genetic basis of ALL
• Many lesions submicroscopic
• Genetic lesions determine risk of disease recurrence
• Specific lesions have roles in outcome in addition to pathogenesis
• Integrated genetic analysis can identify novel targets for therapy

The future

• Current data mostly copy number and gene expression
• Genome wide profiling of CNA, expression, epigenetics
• Arrays → next generation sequencing
• Interrogation of older, and adult cohorts
Acknowledgements

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- Emily Walker

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- Children’s Oncology Group
- NCI, AACR, ASH
**IKZF1 and outcome (ALL0232)**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
<th>IKZF1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETV6-RUNX1</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>MLL</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>TCF3-PBX1</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>BCR-ABL1</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>167</td>
<td>41</td>
</tr>
</tbody>
</table>

- Follow-up: median 3.2 years (0.08-4.6)
- IKZF1 associated with MRD
- IKZF1 associated with EFS in multivariable analysis incorporating MRD

![Graph showing survival analysis with IKZF1 WT and IKZF1 deleted.](attachment:image.png)
## Testing: *IKZF1*

<table>
<thead>
<tr>
<th></th>
<th>MLL</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion</td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td>All gene</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Focal</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>Δ3-6 (Ik6)</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Sequence mutation</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

![Diagram of IKZF1 gene with exon 3-6](image)
Testing – *IKZF1*

- Microarray: Genome wide (SNP 6.0) or targeted (*IKZF1*, other lesions)?
Testing – *IKZF1* and JAK

- **FISH**
- **Exon specific qPCR**
  - Robust, validated
- **DNA PCR for *IKZF1* D3-6**
  - Extremely conserved breakpoints
  - Qualitative – robust
  - Quantitative – ?role
- **RNA PCR for *IKZF1* D3-6**
- **JAK mutation detection**
  - Sanger sequencing
  - Screening – HRM, DHPLC (WAVE)