FAK inhibitors reverse the stromal adhesion phenotype of Ikaros-mutant B-ALL, induce apoptosis, and synergize with ABL1 TKIs: A new paradigm for pathogenesis & therapy of high-risk B-ALL

Ila Joshi
Cutaneous Biology Research Center
Massachusetts General Hospital, Charlestown, MA

56th ASH Annual Meeting and Exposition
San Francisco, CA
Molecular genetic landscape of human precursor B-cell leukemia

- Mutations in transcription factors regulating B-lymphoid development (PAX5, E2A, EBF1, IKZF1) are frequent in B-ALL (Nature 2007; 446:758).

- Deletions and DN mutations in IKZF1 (IKAROS) are extremely frequent (~85%) in Ph+ B-ALL (Nature 2008; 453:110).

- IKZF1 mutations are an independent poor prognostic factor in B-ALL (NEJM 2009; 360:470).

- “Ph–like” B-ALL is characterized by a GEP similar to Ph+ B-ALL and frequent activation of other TKs (JAK2, ABL1/2, PDGFRB, EPOR), and most have IKZF1 mutations (Cancer Cell 2012; 22:153 and NEJM 2014;371:1005).
Key questions in the pathogenesis of high-risk B-ALL

• How does IKAROS mutation contribute to the pathogenesis of B-ALL? Through differentiation arrest?

• Why does IKZF1 mutant B-ALL have a poor prognosis?

• Can we develop new therapeutic approaches to improve the outcome for these patients?
Role of IKAROS in B-lymphoid differentiation

Genes: stem, lymphoid, myeloid, erythroid

<table>
<thead>
<tr>
<th>Hardy stage</th>
<th>HSC</th>
<th>LMPP</th>
<th>Pre-proB (A)</th>
<th>ProB (B)</th>
<th>Large preB-II (C')</th>
<th>Small preB-II (D)</th>
<th>Immature B (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Kit</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD43</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-7R α</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD24</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B220</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+/-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP-1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD25</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Igα</td>
<td>GL</td>
<td>GL</td>
<td>DJ</td>
<td>V-DJ</td>
<td>VDJ</td>
<td>VDJ</td>
<td></td>
</tr>
<tr>
<td>Igκ</td>
<td>GL</td>
<td>GL</td>
<td>GL</td>
<td>GL</td>
<td>GL/VJ</td>
<td>GL/VJ</td>
<td></td>
</tr>
</tbody>
</table>

SLC (VpreB and λ5)

\[ \mu \text{ HC} \]

CD2 – Cre

CD19 – Cre

IκE5Δ/Δ

Ikzf1 locus

WT

Ik-1

Ik-2

Ik-1ΔE5

Ik-2ΔE5

p38
IkE5^Δ/Δ mice have expansion of polyclonal large pre-B cells without disruption of BM myeloerythroid function

Joshi et al., Nat Immunol 2014;15:294
IkE5^Δ/Δ pre-B cells progress to oligoclonal lymphoblastic leukemia
Adhesion pathways are highly upregulated in IkE5Δ/Δ pre-B cells
IkE5Δ/Δ pre-B cells have upregulated integrins, stromal adhesive phenotype, and activation of FAK.
IkE5ΔΔ mutant pre-B cells are sensitive to a FAK inhibitor in vitro and in vivo.
Ikaros mutation identifies a transient, adherent phase of B-lymphoid development with FAK activation.
Retroviral model of BCR-ABL1 B-leukemogenesis

MSCV retroviral vector

Donors:
Ikaros DN mutant  Wild-type (WT)

harvest BM

Expansion of Pre-B cells
in vitro

Recipient: NSG mice

BCR-ABL1 retroviral transduction

transplantation
BCR-ABL1 cooperates with **Ikzf1** mutation

**Survival (%)**

- **Donor:**
  - **IkE5**\(^{\Delta/\Delta}\)
  - **IkE5**\(^{\Delta/+}\)
  - **WT**

**Time after transplantation (d)**

- 0  7  14  21  28  35

**Adhesion to FN (%)**

- **BCR-ABL1**
  - **WT Fl**
  - **WT Adh**
  - **IkE5**\(^{\Delta/\Delta}\)
  - **IkE5**\(^{\Delta/+}\)
  - **WT**
  - **IkE5**\(^{\Delta/\Delta}\)
FAK inhibitor treatment abolishes stromal adhesion in \textit{lkzf1}-mutant BCR-ABL1$^+$ B-ALL

Biochemical activity of VS-4718:

- FAK EC50 = 26nM
- \% Apoptotic

Graphs showing the effect of VS-4718 on cell adhesion and apoptosis.
**IkE5Δ/Δ BCR-ABL1+ ALLs have stromal-dependent resistance to ABL1 TK inhibitors**

**Graphs showing:****

- **No stroma**
- **OP9 stroma**

**Graph Details:**
- **Y-axis:** Percent of Maximum
- **X-axis:** [Dasatinib] nM
- **Lines and Data Points:**
  - **Ikzf1 WT BCR-ABL1+** (black squares)
  - **Ikzf1 DN BCR-ABL1+** (red triangles)

The graphs illustrate the effect of Dasatinib on cell viability in the presence and absence of stroma, with Ikzf1 WT and Ikzf1 DN cell lines.
ABL1 TKIs synergize with FAK inhibitors against IkE5Δ/Δ BCR-ABL1+ B-ALLs
Studies on primary patient B-ALL samples from ECOG 2993
FAK activation in Ph⁺ B-ALLs with *IKZF1* DN mutation
Human *IKZF1* DN Ph+ B-ALL samples display strong stromal adhesion
IKZF1 DN Ph+ B-ALL show high \textit{in vitro} colony-initiating cell frequency.
Human *IKZF1* DN Ph⁺ B-ALLs exhibit a BM adhesive phenotype *in vivo*
FAK inhibition induces selective apoptosis of human *IKZF1* DN Ph+ B-ALLs in vitro

![Graph showing fold change in Annexin V relative to control with *IKZF1*: WT 14171 and Ik6/+ 19309 for vs-4718 concentration of 1 µm and 3 µm.](image-url)
Combined ABL and FAK inhibition induces apoptosis in human *IKZF1* DN Ph+ B-ALLs in vitro
A new model of high-risk B-ALL

*Ikzf1*-mutant large pre-B (non-malignant)
Next steps

- Currently testing efficacy of FAK inhibitors ± ABL1 TKIs in vivo

- Phase I clinical trial in relapsed/refractory acute leukemia