Introduction

Bruton’s tyrosine kinase (BTK) is a member of the Tec family of non-receptor tyrosine kinases and is predominantly expressed in hematopoietic cells, except in T cells. BTK plays a prominent role in B cell receptor (BCR) signaling and several other pathways, including CXCR4 signaling, which is essential for lymphocyte homing. BTK activation downstream of the BCR leads to proliferation, differentiation, and survival of B cells. Functional BTK is necessary for normal B cell development; defective BTK result in a primary immunodeficiency called X-linked agammaglobulinemia (XLA). Because of the restricted expression and the B cell phenotype in BTK-deficient mice and XLA patients, BTK has become a promising therapeutic target in mature B cell malignancies. Ibrutinib (PCI-32765) is a selective, orally bioavailable, covalent BTK inhibitor currently studied in late stage clinical trials in patients with Chronic Lymphocytic Leukemia (CLL) and other mature B cell malignancies.

BTK Expression in Patient Samples

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BTK Expression in Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Comparison</th>
<th>Expression</th>
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</thead>
<tbody>
<tr>
<td>K562</td>
<td>NC</td>
<td>Low</td>
</tr>
<tr>
<td>H9</td>
<td>NC</td>
<td>Absent</td>
</tr>
<tr>
<td>AML1217</td>
<td>ibrutinib</td>
<td>High</td>
</tr>
<tr>
<td>Kasumi-2</td>
<td>ibrutinib</td>
<td>Low</td>
</tr>
<tr>
<td>RCH-ACV</td>
<td>ibrutinib</td>
<td>Low</td>
</tr>
<tr>
<td>VPC-78</td>
<td>ibrutinib</td>
<td>Low</td>
</tr>
</tbody>
</table>

![Figure 1. BTK expression in B-ALL cell lines.](image)

Effect of ibrutinib on Cell Lines

- **A**: Ibrutinib concentration, µM
- **B**: BTK expression
- **C**: Ibrutinib suppressed growth of several B-ALL cell lines.
- **D**: Summarized results of 3 independent XTT proliferation assays.

![Figure 2. Ibrutinib expression in B-ALL patient samples.](image)

Conclusions

- The majority of B-ALL cell lines express BTK; some of them also exhibit BTK phosphorylation.
- BTK is present in primary B-ALL samples.
- Ibrutinib effectively occupies BTK in B-ALL cell lines at the concentration of 0.01 µM (BTK occupancy probe assay).
- With a few exceptions, B-ALL cell lines display decreased proliferation in the presence of ibrutinib. RCH-ACV and SMS-SB are the most sensitive to the drug.
- Changes in viability in all cases are minor.
- In some cases primary B-ALL cells show 10 to 25% reduced viability after ibrutinib treatment.
- Further experiments are needed to find a suitable marker for ibrutinib sensitivity.

Disclosures

- Dr. Susan O’Brien has research support from Pharmacyclics.
- Dr. Joseph J. Buggy is an employee and shareholder of Pharmacyclics.
- Dr. Jan A. Burger is a consultant and has research funding from Pharmacyclics.

![Figure 3. Ibrutinib occupied the active site of BTK in B-ALL cell lines.](image)

![Figure 4. Ibrutinib suppressed growth of several B-ALL cell lines.](image)

![Figure 5. Ibrutinib reduced primary B-ALL cells survival in part of the cases.](image)