SNS-062 demonstrates efficacy in chronic lymphocytic leukemia *in vitro* and inhibits C481S mutated Bruton tyrosine kinase

Catherine A. Fabian1, Sean D. Reiff2, Daphne Guinn3, Amy Lehman1, Linda Neuman3, Judith A. Fox4, Wendy Wilson3, John C. Byrd1, Jennifer A. Woyach1, and Amy J. Johnson1.
1The Ohio State University, Columbus, OH; 2Georgetown University, Washington, D.C.; 3Sunesis Pharmaceuticals, South San Francisco, CA.

Abstract #: 1207

**Background**

- B-cell receptor signaling (BCR) is exceptionally active in chronic lymphocytic leukemia (CLL) and is vital for the proliferation and survival of CLL cells. BTK is upregulated at the RNA and protein level in CLL, implicating its essential role in BCR signaling and pathogenesis of B-lymphoid malignancies.
- Ibrutinib, an irreversible, covalent BTK inhibitor, is an effective targeted therapy for patients with CLL.
- A subset of patients acquire resistance to ibrutinib and progress due to BTK C481S mutation, as the C481 residue is required for covalent bond formation.
- SNS-062 is a noncovalent, reversible BTK inhibitor that inhibits signaling through the BCR pathway. SNS-062 does not require interaction with BTK C481 for inhibitory activity. SNS-062 has improved PK properties over ibrutinib, including greater bioavailability and a longer half-life.
- SNS-062 may offer an alternative treatment option to patients with acquired resistance to ibrutinib.
- In these studies, we sought to characterize SNS-062 in preclinical models of CLL.

**Methods**

- Primary CLL B-cells were isolated from whole blood of consented patients by Ficoll density centrifugation and Rosette-sep negative selection.
- BCR signaling and BTK inhibition in primary CLL cells and in X-linked Agammaglobulinemia (XLA) human cell lines were investigated by immunoblot following 1 hour treatment with SNS-062 and ibrutinib and 15 minute stimulation with α-IgM in primary CLL cells.
- Measurement of kinase activity in recombinant BTK WT or BTK C481S was performed in a FRET kinase assay.
- CD40 and CD86 surface expression on patient CLL cells was evaluated via flow cytometry subsequent to sustained 3.2uM CpG stimulation and 48 hour treatment with SNS-062 and ibrutinib.
- 7-AAD was used to measure patient CLL cell viability in HS5-GFP stromal co-culture after 49 hour treatment with SNS-062 and ibrutinib.
- CD40 and CD86 surface expression on patient CLL cells was evaluated via flow cytometry subsequent to sustained 3.2uM CpG stimulation and 48 hour treatment with SNS-062 and ibrutinib.
- Annexin V and propidium iodide flow cytometry was used to measure primary patient CLL cell viability after 48 hour treatment with increasing doses of SNS-062 and 1 μM ibrutinib.

**Results**

**Primary Patient:** SNS-062 demonstrates dose-dependent BTK inhibition

**XLA Cells:** SNS-062 inhibits BTK activation in a dose-dependent manner. BTK inhibition by SNS-062 at 1 μM concentration was comparable to inhibition by 1 μM Ibrutinib condition. *p<0.01

**Inhibitory Activity of SNS-062**

<table>
<thead>
<tr>
<th>Kinase</th>
<th>IC50 (nM)</th>
<th>SNS-062</th>
<th>Ibrutinib</th>
<th>Acalabrutinib</th>
<th>NS-062</th>
<th>#</th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>BTK</td>
<td>3</td>
<td>14</td>
<td>23</td>
<td>474</td>
<td>224</td>
<td>8</td>
<td>84</td>
</tr>
<tr>
<td>TEC</td>
<td>10.7</td>
<td>78</td>
<td>5.0</td>
<td>ND</td>
<td>0.5</td>
<td>84</td>
<td>30</td>
</tr>
<tr>
<td>BLK</td>
<td>33</td>
<td>32.2</td>
<td>171</td>
<td>171</td>
<td>ND</td>
<td>90</td>
<td>664</td>
</tr>
<tr>
<td>TXK</td>
<td>5.6</td>
<td>6.6</td>
<td>66</td>
<td>66</td>
<td>ND</td>
<td>66</td>
<td>66</td>
</tr>
</tbody>
</table>

*SNS-062 inhibits BTK activation in a dose-dependent manner. BTK inhibition by SNS-062 at 1 μM concentration was comparable to inhibition by 1 μM Ibrutinib condition. *p<0.01

**Conclusions**

- Unlike ibrutinib, SNS-062 inhibition of BTK signaling is unaffected by the presence of the C481S mutation and may address acquired resistance to covalent BTK inhibitors.
- SNS-062 decreases surface expression of B-cell activation markers and patient CLL cell viability. These effects are comparable to ibrutinib.
- SNS-062 diminishes stromal cell protection in patient CLL cells, revealing its abilities to abrogate protection from the microenvironment.
- The promising nonclinical activity of SNS-062 supports further investigation and advancement into clinical trials. A Phase 1b/2 study in patients with advanced B-cell malignancies will initiate within the first half of 2017.