Introduction

- Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia characterized by clonal proliferation of malignant B-lymphocytes.
- Although standard-of-care agents are well tolerated in CLL patients with certain genetic subsets of the disease continue to display poor response to these therapeutic regimens.
- Menin is an epigenetic protein that drives oncogenic function through transcriptional regulation directed by interactions with various protein partners. In the B-cell maturation pathway, menin regulates a distinct set of gene targets\(^1\).

Methods

- CLL models from BTX experienced patients were cultured ex vivo in the presence of BMF-219, reversible BTKI pirtobrutinib, or venetoclax for 6 days to assess the anti-leukemic activity of the compounds.
- RNA-seq was conducted 24 hours after BMF-219 treatment on the Illumina NovaSeq 500 platform and analysis was performed using Bioconductor (https://bioconductor.org). Differential gene expression was determined using the edgeR function.

Results

- BMF-219 exerts dose-dependent reduction of menin target genes in CLL patient samples
- BMF-219 demonstrates superior potency and ability to achieve >99% growth inhibition in ex vivo cultured CLL patient specimens in comparison with reversible BTK inhibitor, pirtobrutinib.
- Differential gene expression analysis of BMF-219 treated CLL patient samples revealed reduction of MEN1 and BCL2 expression in both models.
- BMF-219 exerted a dose-dependent reduction of NOTCH1 and MYC in the patient model with a complex cytoskeletal background.
- Gene set enrichment analysis (GSEA) highlighted novel molecular pathways altered by BMF-219 belonging to cell adhesion and cytokine receptor signaling including the CXC chemokine family.
- Other notable pathways downregulated by BMF-219 included autophagy function pathways such as Type 1 Diabetes Mellitus, with reduction of IRE1.
- Collectively, these data demonstrate the mechanistic impact of BMF-219 on key gene targets and molecular pathways modulated by covalent menin inhibition, further highlighting its potential as a novel therapeutic agent in CLL compared to new investigational drugs currently in clinical development and established standards-of-care for CLL.

Table 1. Clinical Profiles of CLL Patient Samples and Response to BMF-219

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mutation</th>
<th>Cytogenetics</th>
<th>Prior Treatment</th>
<th>IC(_{50}) (nM)</th>
<th>Max Growth Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-303</td>
<td>TP53</td>
<td>N/A</td>
<td>BMF-219</td>
<td>0.25</td>
<td>&gt;10</td>
</tr>
<tr>
<td>BM-306</td>
<td>TP53</td>
<td>Normal</td>
<td>BMF-219</td>
<td>0.36</td>
<td>&gt;10</td>
</tr>
<tr>
<td>BM-308</td>
<td>None or N/A</td>
<td>BMF-219</td>
<td>BMF-219</td>
<td>0.27</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

Figure 1. Growth inhibition of CLL patient-derived samples treated with BMF-219, venetoclax, or pirtobrutinib. CLL patient samples were cultured ex vivo in the presence of BMF-219, venetoclax or pirtobrutinib for 24 hours and cell viability was measured by C47 Titer Glu. Each data point represents the average of at least two replicate values. Treating IC\(_{50}\) determined as a standard-experiment (BMF-303: 29 nM; BMF-308: 24 nM; BMF-306: 17 nM). Clinical profiles of each patient sample, including prior therapy, and IC\(_{50}\) values for each compound are summarized in Table 1.

Figure 2. Gene expression of BMF-219 treated CLL patient samples. CLL patient samples were treated with BMF-219 (250 nM or 500 nM) for 24 Hours. Counts per million (CPM) normalized values for MEN1 and BCL2 in model BM-306 (A, B) MEN1 and BCL2 in model BM-306 (E, D), and NOTCH1 and MYC expression in model BM-306 (F) were calculated using the cpm function in the edgeR package\(^{2}\) with log2. Each bar represents the average of at least two replicate samples. Housekeeping genes, GAPDH, was not affected by BMF-219 treatment in both models.

Figure 3. BMF-219 downregulates cell adhesion, cytokine signaling and autoimmune pathways in CLL patient samples. Gene set enrichment analysis (GSEA) using the GSEA R package was calculated using the log, fold change between BMF-219 vs. vehicle treated Model BM-306 (A, B), and BMF-219 in vehicle treated model BM-306 (C). Differential expression comparison was used to rank genes. Bar shows the genes ranked by log fold change along the x-axis. Genes grouped in red are more expressed in the first group (BMF-219 treated) and genes grouped in blue are more expressed in the second group (0.1% DMSO). The green line depicts the enrichment score. Notable genes reduced include the CXC chemokine receptors (B) and IL8 (C).

References
