# Covalent menin inhibitor, BMF-219, impacts key gene signatures and molecular pathways in Chronic Lymphocytic Leukemia patient-derived models

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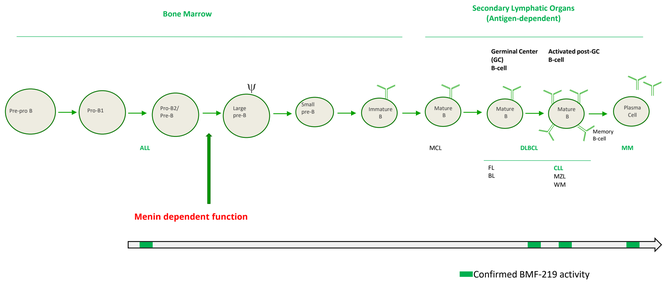
## Introduction

- 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].




- We previously described the potent activity of BMF-219, a selective covalent oral menin inhibitor, against a diverse panel of CLL patient specimens with various cytogenetic and mutational backgrounds, including TP53 and NOTCH1 mutations[2].
- BMF-219 is currently in a Phase 1 first-in-human dose finding study for adult patients with Acute Leukemia (AL), Diffuse Large B-cell Lymphoma (DLBCL), Multiple Myeloma (MM), and Chronic Lymphocytic Leukemia (CLL)/ Small Lymphocytic Lymphoma (SLL) (COVALENT-101: CT Identifier NCT05153330).
- Here, we provide insights into the molecular impact of BMF-219 in CLL patient samples, as revealed through gene expression profiling of CLL specimens from Bruton Tyrosine Kinase (BTK) inhibitor experienced patients that represent clinical profiles of TP53-mutated and complex cytogenetic backgrounds.

## Methods

- CLL models from BTKi experienced patients were cultured *ex vivo* in the presence of BMF-219, reversible BTKi pirtobrutinib, or venetoclax for 6 days to assess the antileukemic activity of the compounds.
- RNA-seq was conducted 24 hours after BMF-219 treatment on the Illumina NextSeq 550 platform and analysis was performed using Pluto (https://pluto.bio). Differential gene expression was determined using the edgeR function.

## BMF-219 achieves > 98% cell killing against BTKi-resistant CLL patient samples

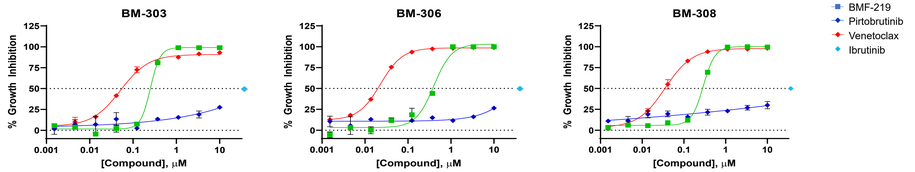



**Table 1. Clinical Profiles of CLL Patient Samples and Response to BMF-219**

| Sample | Mutation | Cytogenetics | Prior Treatment | BMF-219 | | Pirtobrutinib | |
|---|---|---|---|---|---|---|---|
| | | | | $IC_{50}$ (μM) | Max Growth Inhibition (%) | $IC_{50}$ (μM) | Max Growth Inhibition (%) |
| BM-303 | TP53 | N/A | Ibrutinib (responded), (post-collection: ibrutinib and venetoclax-responded, then progressed) | 0.25 | 100 | >10 | 27.5 |
| BM-306 | TP53 | Normal | Ibrutinib (responded, no progression) | 0.36 | 100 | > 10 | 26.5 |
| BM-308 | None or N/A | 46~47, XY, del(6)(q13q25), dic(7;21)(q31;p13), add(11)(q13), del(13)(q12q14), +2mar, inc [cp4]/46, XY [3] | Rituximab/Ibrutinib (responded, continuing) | 0.27 | 99 | > 10 | 30 |

**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 6 days and cell viability was measured by Cell Titer Glo. Each data point represents the average of at least two replicate values. Ibrutinib $IC_{50}$ determined as a standalone experiment (BM-303: 29 μM, BM-306: 24 μM, BM-308: 27 μM). Clinical profiles of each patient sample, including prior therapy, and $IC_{50}$ values for each compound are summarized in **Table 1.**

## BMF-219 achieves > 98% cell killing against BTKi-resistant CLL patient samples

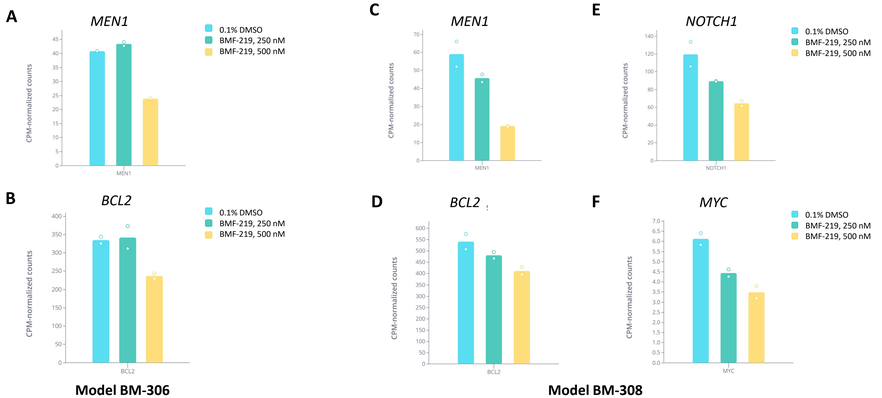



**Figure 2. Gene expression of BMF-219 treated CLL patient samples.** CLL patient samples were treated with BMF-219 (250 nM or 500 nM) for 24 hours. Counts per million (CPM) normalized values for *MEN1* and *BCL2* in model BM-306 **(A, B)**, *MEN1* and *BCL2* in model BM-308 **(C, D),** and *NOTCH1* and *MYC* expression in model BM-308 **(E,F)** were calculated using the cpm function in the *edgeR* R package[1] with log=F. Each bar represents the average of at least two replicate samples. Housekeeping gene, *GAPDH*, was not affected by BMF-219 treatment in both models.

## BMF-219 downregulates cell adhesion, cytokine signaling and autoimmune pathways in CLL patient samples

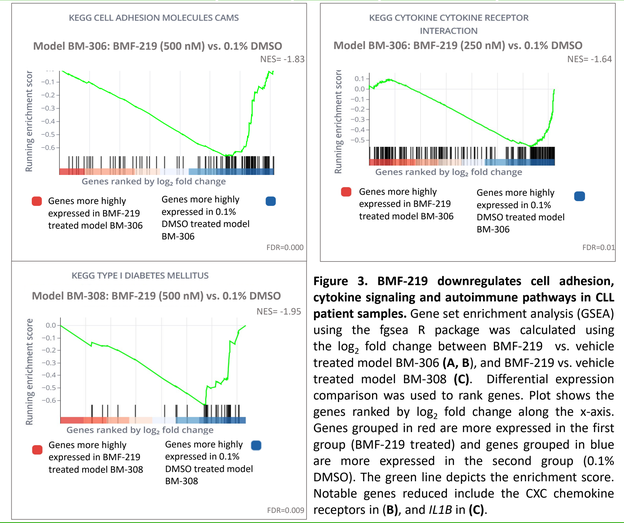



**Figure 3. BMF-219 downregulates cell adhesion, cytokine signaling and autoimmune pathways in CLL patient samples.** Gene set enrichment analysis (GSEA) using the fgsea R package was calculated using the $log_2$ fold change between BMF-219 vs. vehicle treated model BM-306 **(A, B)**, and BMF-219 vs. vehicle treated model BM-308 **(C)**. Differential expression comparison was used to rank genes. Plot shows the genes ranked by $log_2$ 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 in **(B)**, and *IL1B* in **(C)**.

## Conclusions

- BMF-219 demonstrated 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 cytogenetic 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 autoimmune function pathways such as Type 1 Diabetes Mellitus, with reduction of *IL1B*.
- 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 standard-of-care agents for CLL.

### References

1. Li, BE., Gan, T., Meyerson, M., et al. Distinct pathways regulated by menin and by MLL1 in hematopoietic stem cells and developing B cells. *Blood* (2013) 122 (12): 2039–2046.
2. Somanath, P., Lu, D., Law, B. et al. Preclinical activity of irreversible Menin inhibitor, BMF-219, in chronic lymphocytic leukemia. J Clin Oncol 40, 2022 (suppl 16; abstr 7541).

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