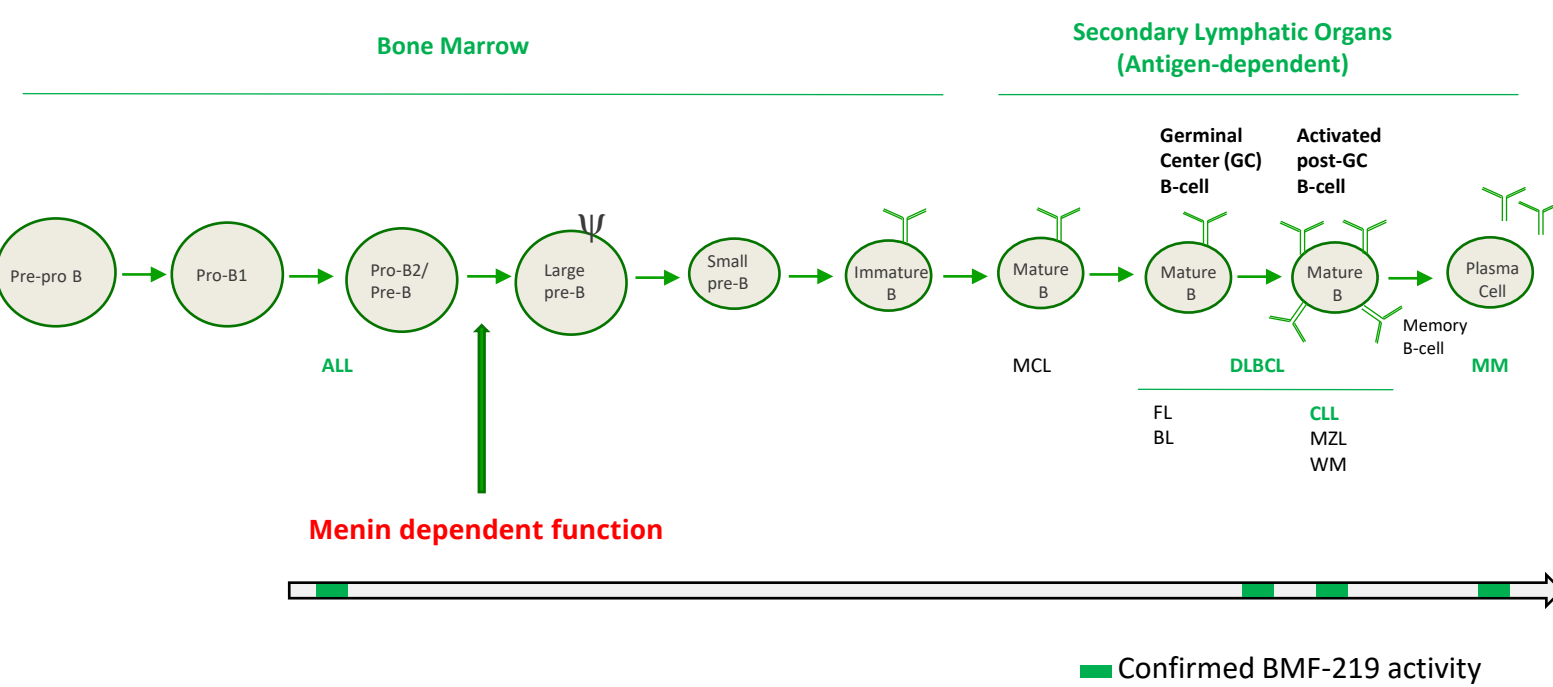


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Introduction

- 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 targets¹.



- 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 mutations².
- 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 Novoseq 500 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

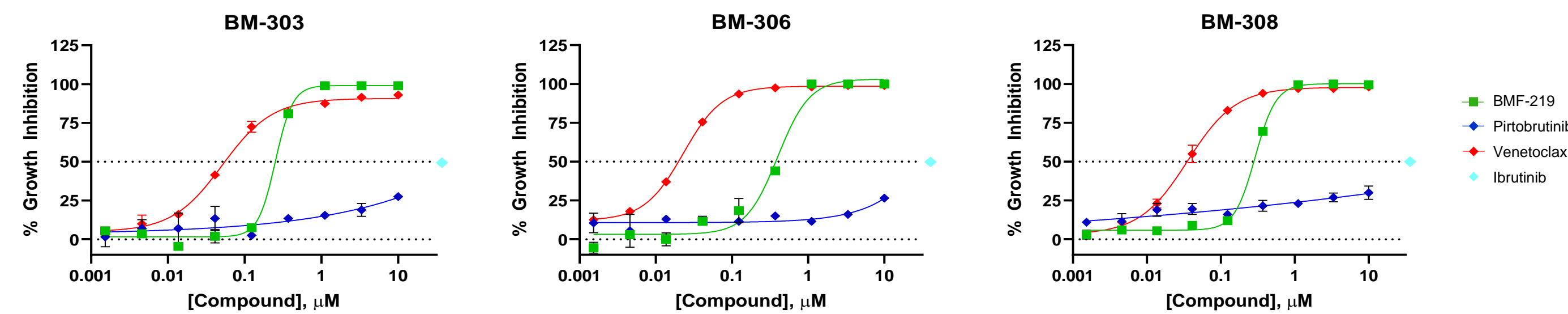


Table 1. Clinical Profiles of CLL Patient Samples and Response to BMF-219

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 IC₅₀ 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 IC₅₀ values for each compound are summarized in **Table 1**.

BMF-219 exerts dose dependent reduction of menin target genes in CLL patient samples

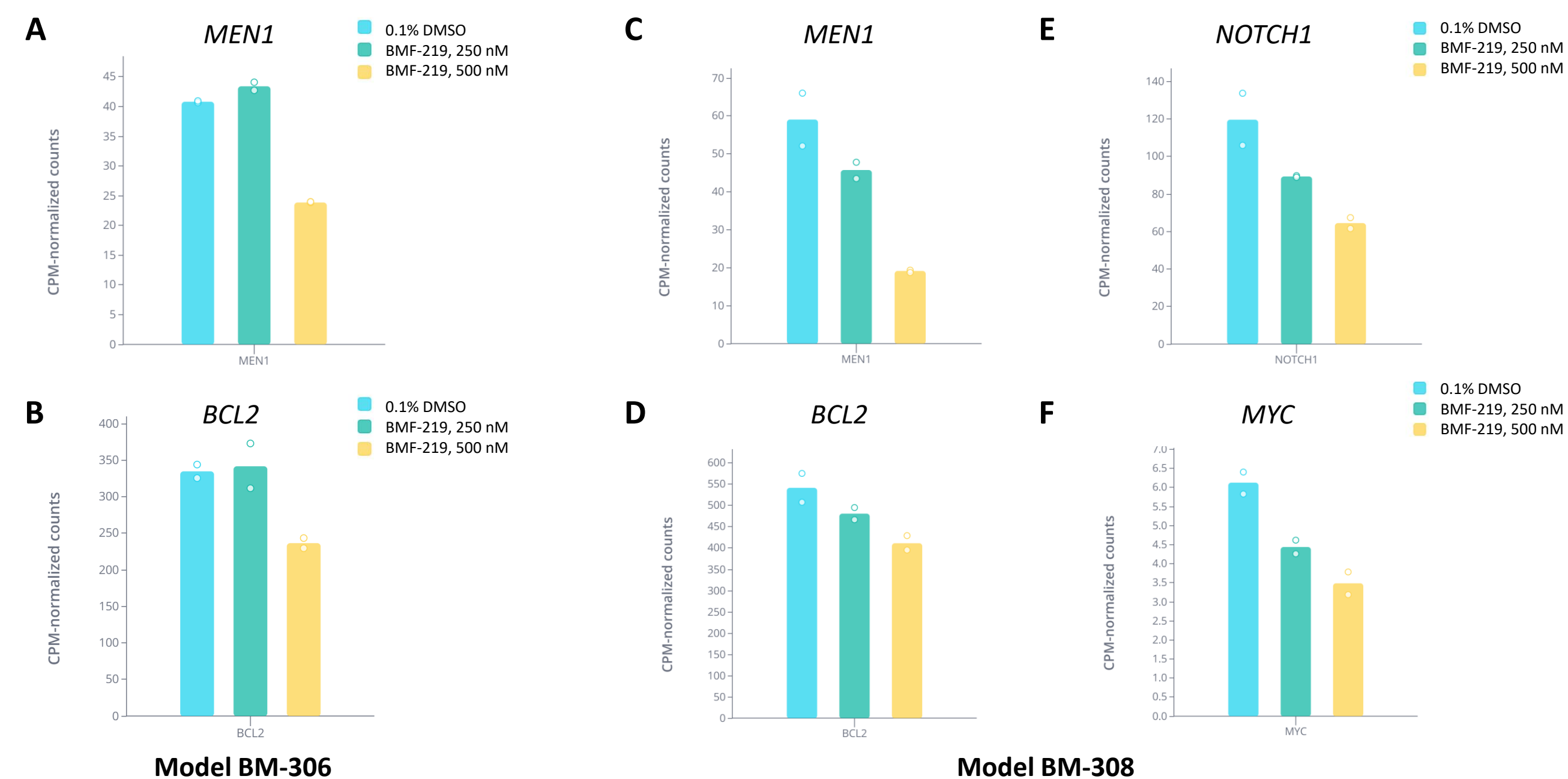


Figure 2. Gene expression of BMF-219 treated CLL patient samples. CLL patient samples were treated with BMF-219 (250 nM or 500 nM) for 24 hours. Counts per million (CPM) normalized values for *MEN1* and *BCL2* in model BM-306 (A, B), *MEN1* and *BCL2* in model BM-308 (C, D), and *NOTCH1* and *MYC* expression in model BM-308 (E, F) were calculated using the *cpm* function in the *edgeR* R package¹ 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

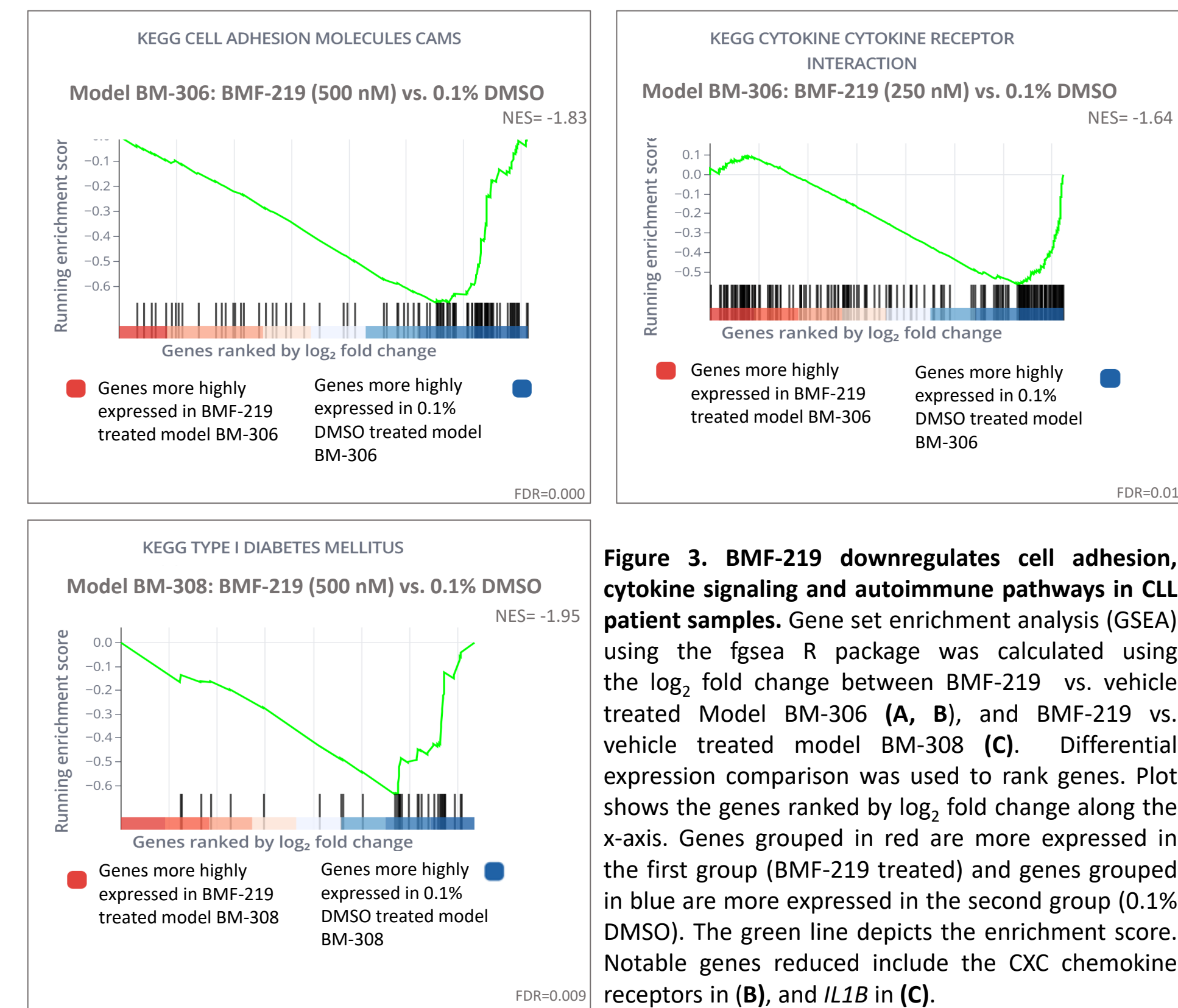


Figure 3. BMF-219 downregulates cell adhesion, cytokine signaling and autoimmune pathways in CLL patient samples. Gene set enrichment analysis (GSEA) using the *fgsea* R package was calculated using the log₂ fold change between BMF-219 vs. vehicle treated Model BM-306 (A, B), and BMF-219 vs. vehicle treated model BM-308 (C). Differential expression comparison was used to rank genes. Plot shows the genes ranked by log₂ 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

- 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.
- 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).