Anti-tumor Activity of Covalent Menin Inhibitor, BMF-219, in High Grade B-Cell Lymphoma and Multiple Myeloma Preclinical Models

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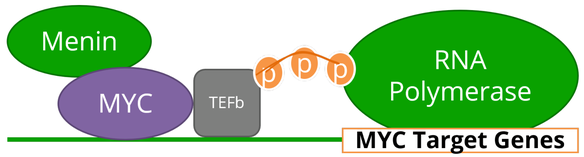
INTRODUCTION

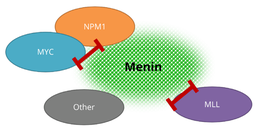
- Menin is a scaffold protein that drives oncogenic function through its regulation of genes such as *HOXA9*, with distinct effects on transcription that are directed by various cofactors. A recent study reported that knockdown of *HOXA9* resulted in marked growth inhibition of multiple myeloma (MM) cells (Chapman et al., 2017).
- Double/Triple Hit Lymphoma (DHL/THL) and Double Expresser Lymphoma (DEL) are high-grade B-cell lymphomas (HGBL) that exhibit low responses to standard therapeutic regimens resulting in poor prognosis.
- DHL harbor translocations in MYC and BCL2 or BCL6, THL contain translocations in MYC/BCL2/BCL6, and DEL are characterized by high expression of MYC and BCL2.
- We previously reported the ability of irreversible menin inhibitor, BMF-219, to modulate MYC expression and exhibit high potency against DHL Diffuse Large B-Cell Lymphoma (DLBCL) preclinical models (Somanath et al., 2021).



Source: Wu, G. et al. Menin enhances c-Myc-mediated transcription to promote cancer progression. Nat. Commun. 8, 15278 (2017).

- Here, we demonstrate the anti-tumor activity of BMF-219 in MM and HGBL preclinical models harboring various mutational backgrounds.

Mechanism of Action



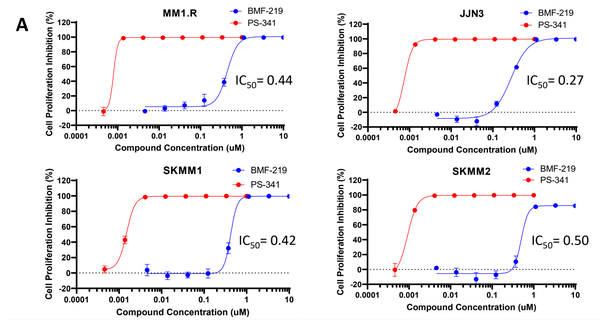
Target Patient Population

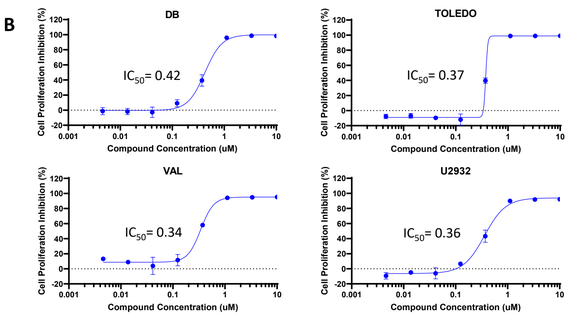
- MLL — MLL-r Acute Leukemia
- NPM1 — NPM1 mutant Acute Leukemia
- MYC — DHL / DEL DLBCL; Multiple Myeloma; KRAS mutant (CRC, Lung, Pancreatic)
- Other — Liquid and Solid Tumors

METHODS

- MM and DLBCL cell lines were cultured in the presence of BMF-219 or bortezomib (PS-341) for 4 days and cell proliferation was measured by Cell Titer Glo.
- Patient-derived MM patient derived BMMCs and DLBCL PDX models were cultured *ex vivo* in the presence of BMF-219 or PS-341 for 6 days and cell proliferation was measured by Cell Titer Glo.
- MM and DLBCL cell lines were cultured in the presence of BMF-219 or clinical reversible menin inhibitors for 14 hours. Menin protein expression was measured by the Wes system and analyzed using the Compass software (automated western blotting, Protein Simple). Signal was normalized to GAPDH and referenced to DMSO control.

BMF-219 exerts >99% lethality against MM and DLBCL cell lines





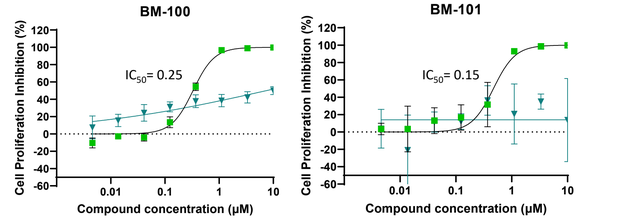
MM Cell Line	Translocation	Mutation	Average % Max Inhibition by BMF-219	Average IC_{50} ± Standard Deviation (μM)
MM1.S	t(14;16)	KRAS G12A	99.5	0.47 ± 0.17
MM1.R	t(14;16)	KRAS G12A	99.6	0.46 ± 0.17
SKMM1	t(14;20)	NRAS G12A	99.2	0.47 ± 0.05
SKMM2	t(11;14)	TP53	80.2	0.65 ± 0.15
JJN3	t(14;16)	NRAS Q61K	99.2	0.29 ± 0.02
OPM2	t(4;14)	TP53	98.4	0.55

DLBCL Cell Line	Category	Translocation	Average % Max Inhibition by BMF-219	Average IC_{50} ± Standard Deviation (μM)
DB	DHL	MYC/BCL2	98.5	0.41 ± 0.07
Toledo	DHL	MYC/BCL2	98.8	0.31 ± 0.07
DOHH2	DHL	MYC/BCL2	99.7	0.32 ± 0.03
VAL	THL	MYC/BCL2/BCL6	97.1	0.27 ± 0.07
U2932	DEL-ABC	MYC/BCL2 Overexpression	92.4	0.37 ± 0.01
SUDHL8	GCB	-	99.6	0.60 ± 0.21
Pfeiffer	GCB	-	99.6	0.17 ± 0.04
OCI-LY7	GCB	-	99.6	0.65 ± 0.26

Potent killing activity of BMF-219 at clinically relevant concentrations in representative MM and DLBCL cell lines. (A). MM1.R, JJN3, SKMM1, SKMM2 and OPM2 cell lines and (B). DHL (DB, Toledo, DOHH2), THL (VAL), DEL (U2932), and GCB (SUDHL8) subtypes were cultured in the presence of BMF-219 (blue) or PS-341 (red) for 4 days. Average IC_{50} values of at least two experiments, maximal percentage cell killing, and cytogenetic background cell lines are summarized in the table.

BMF-219 exerts pronounced lethality in DLBCL PDX models *ex vivo*

THL- Responded, then progressed on R-EPOCH | **MYC Amplified- Responded, then progressed on R-CHOP**



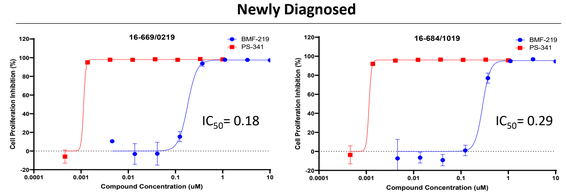
	Growth Inhibition IC_{50} (μM)	% Max Inhibition	Growth Inhibition IC_{50} (μM)	% Max Inhibition
Treatment	BM100		BM101	
BMF-219	0.250	100	0.151	100
Clinical Reversible	6.31	50	> 10	30

Growth inhibition of patient-derived DLBCL Triple Hit Lymphoma (THL) and MYC-amplified PDX samples treated with BMF-219 or clinical reversible menin inhibitor after 6 days of treatment. IC_{50} values and maximal percentage cell killing are summarized in the table.

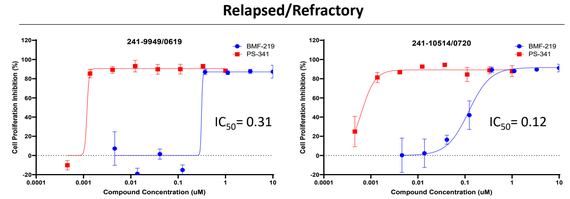
BMF-219 dramatically reduces growth of both newly diagnosed and R/R MM patient specimens

Multiple Myeloma Specimen ID	Stage at Diagnosis	Treatment Status	Prior Therapy and Response	Translocation
16-669/0219	IIIA	Newly Diagnosed	None	No data
16-684/1019	IIIA	Newly Diagnosed	None	No data
241-9949/06-19	IIIA	Refractory	VCD N4 (resistant)	p53 deletion
241-10514/0720	IIIA	Refractory	VCD N 4 (responded)	p53 deletion-negative
			High dose CPH (SC-mobilization) (responded)	
			Consolidation (AutoSCT, double transplant)	
			Bortezomib- maintenance (resistant)	
			RVD #4 (resistant)	
			PRD #4 (resistant)	

Newly Diagnosed

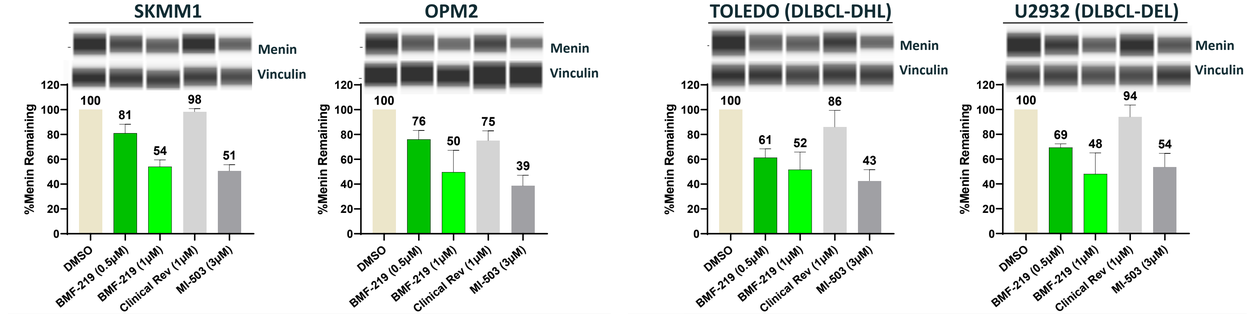


Relapsed/Refractory



Growth inhibition of newly diagnosed (A-B) and R/R (C-D) MM patient-derived bone marrow mononuclear cells (BMMCs) after 6 days of treatment with BMF-219 (blue) or PS-341 (red). Clinical profiles of MM patient-derived BMMC specimens are summarized in the table.

BMF-219 exerts pronounced decrease in menin protein expression in MM and DLBCL cell lines



% Cell Death	SKMM1					OPM2				
	BMF-219 (0.4μM)	BMF-219 (0.5μM)	BMF-219 (1μM)	Clin Rev (1μM)	MI-503 (3μM)	BMF-219 (0.4μM)	BMF-219 (0.5μM)	BMF-219 (1μM)	Clin Rev (1μM)	MI-503 (3μM)
72 hr	27	-	86	4	33	22	-	80	3	21
14 hr	-	15	25	0	13	-	8	57	0	14

% Cell Death	TOLEDO					U2932				
	BMF-219 (0.4μM)	BMF-219 (0.5μM)	BMF-219 (1μM)	Clin Rev (1μM)	MI-503 (3μM)	BMF-219 (0.4μM)	BMF-219 (0.5μM)	BMF-219 (1μM)	Clin Rev (1μM)	MI-503 (3μM)
72 hr	32	-	97	0	35	29	-	86	3	34
14 hr	-	18	12	0	11	-	19	36	0	7

BMF-219 reduces menin protein in MM and DLBCL cells. Quantitation of menin protein expression in SKMM1, OPM2, Toledo and U2932 cell line treated with BMF-219, clinical reversible menin inhibitor or preclinical reversible menin inhibitor, MI-503, for 14 hours. Average menin protein expression are of 3 independent experiments. WES blot is a representative from 1 single experiment. Cells were cultured in the presence of menin inhibitors for 72hr or 14hr. Average % cell killing treated at 72hr and 14hr are from 2 independent experiment.

CONCLUSIONS

- BMF-219 achieved >99% cell lethality in MM cell lines with RAS mutations with IC_{50} values between 0.3 μM and 0.5 μM.
- BMF-219 demonstrated single-agent efficacy (IC_{50} values between 0.1 μM and 0.3 μM) against a panel of newly diagnosed and R/R *ex vivo* MM samples, including a p53-deleted clinical profile.
- BMF-219 exhibited high potency as a single agent against DHL, THL and DEL DLBCL cell lines, with IC_{50} values of 0.3 μM and 0.4 μM, respectively.
- In *ex vivo* studies, BMF-219 was highly effective against R-CHOP and R-EPOCH refractory patient samples with THL and MYC-amplified genetic backgrounds.
- BMF-219 was multi-fold more potent and exerted dramatically greater growth inhibition compared to clinical reversible menin inhibitors in DLBCL patient-derived *ex vivo* samples.
- BMF-219 induces reduction of menin protein levels across MM and DLBCL cell lines. This reduction however appears to be transient. An incubation time of 14 hours may not be a good predictor of cellular growth inhibition.

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