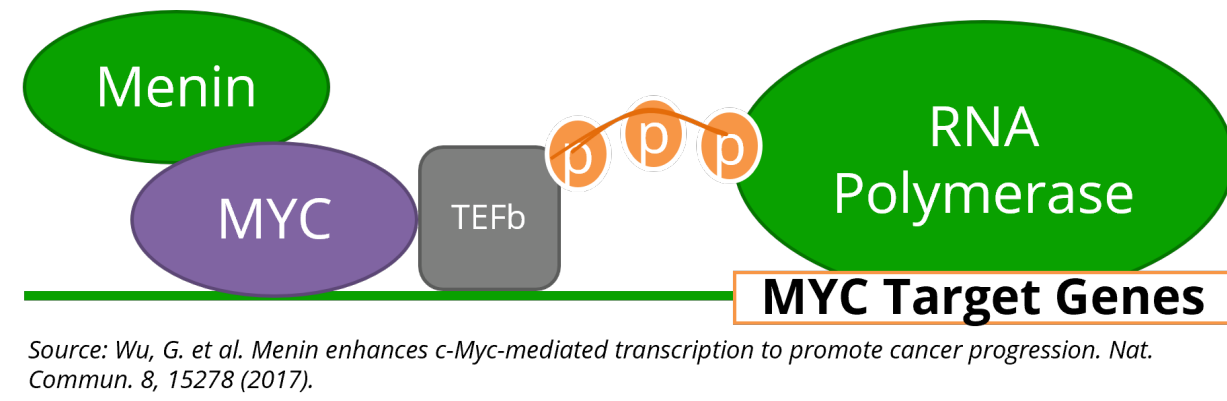
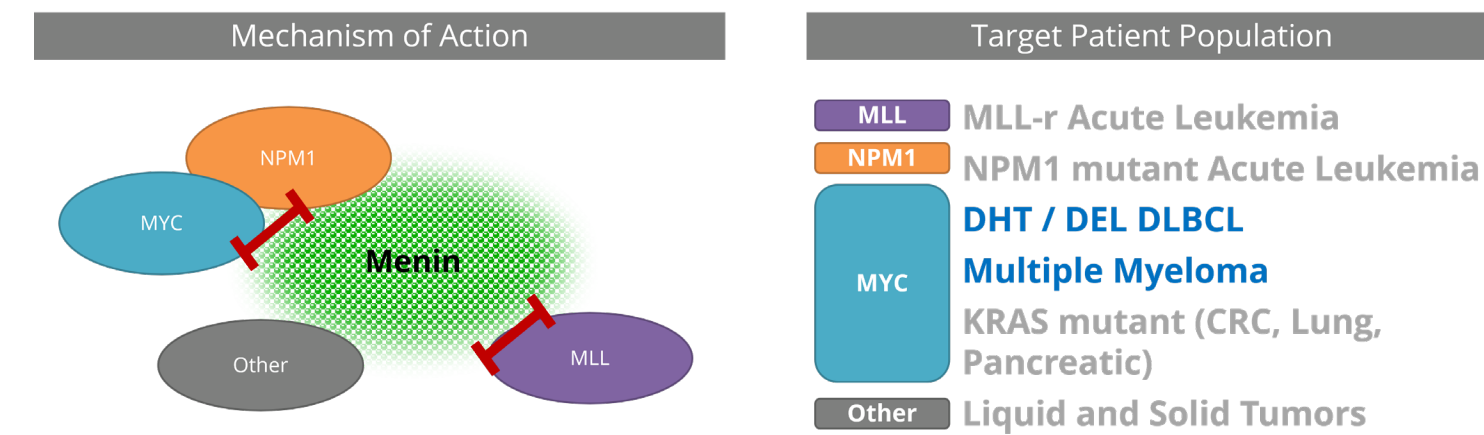


INTRODUCTION

- 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.
- 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).
- 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).



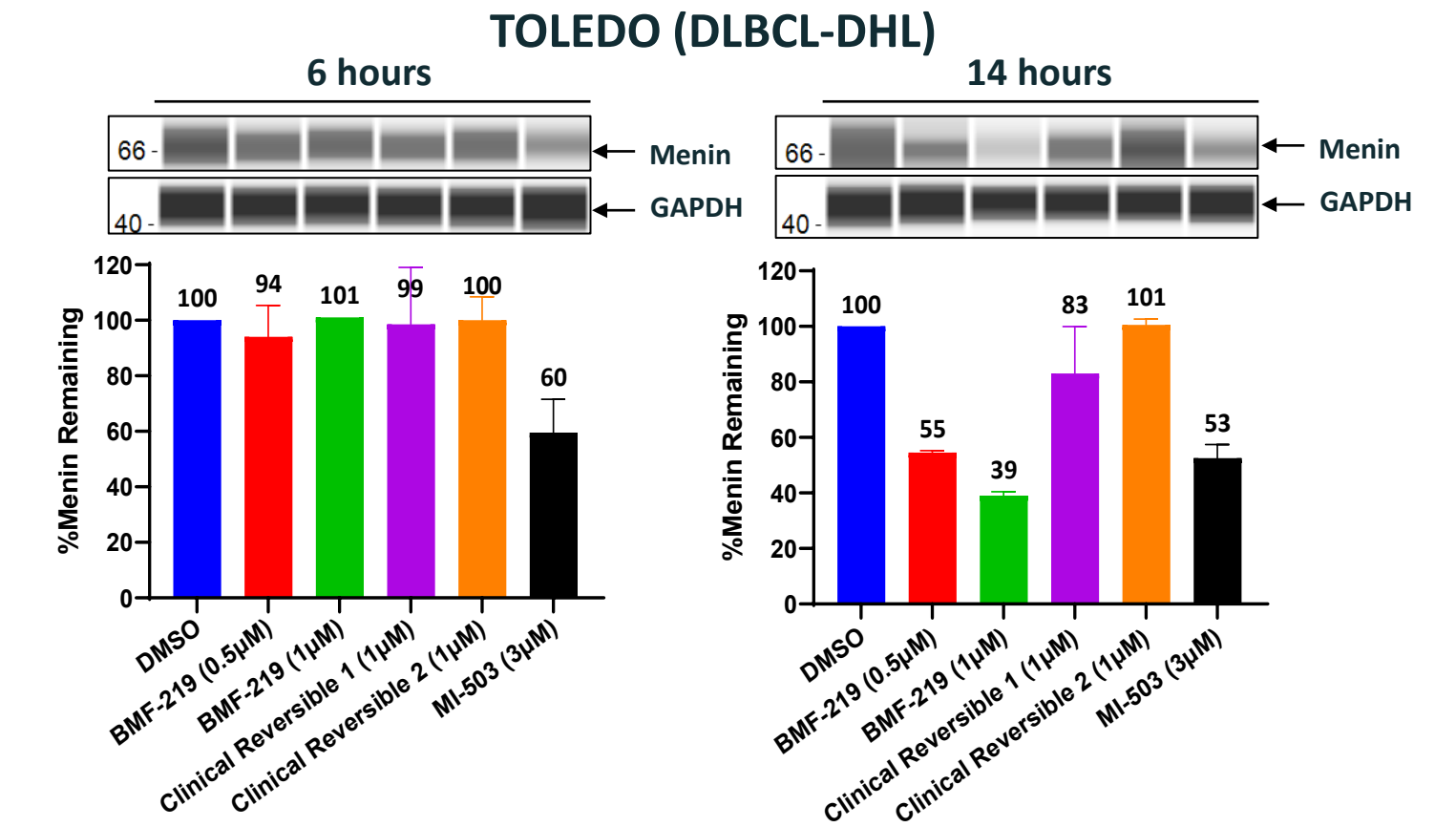
- Here, we demonstrate the anti-tumor activity of BMF-219 in HGBL and MM preclinical models harboring various mutational backgrounds.



METHODS

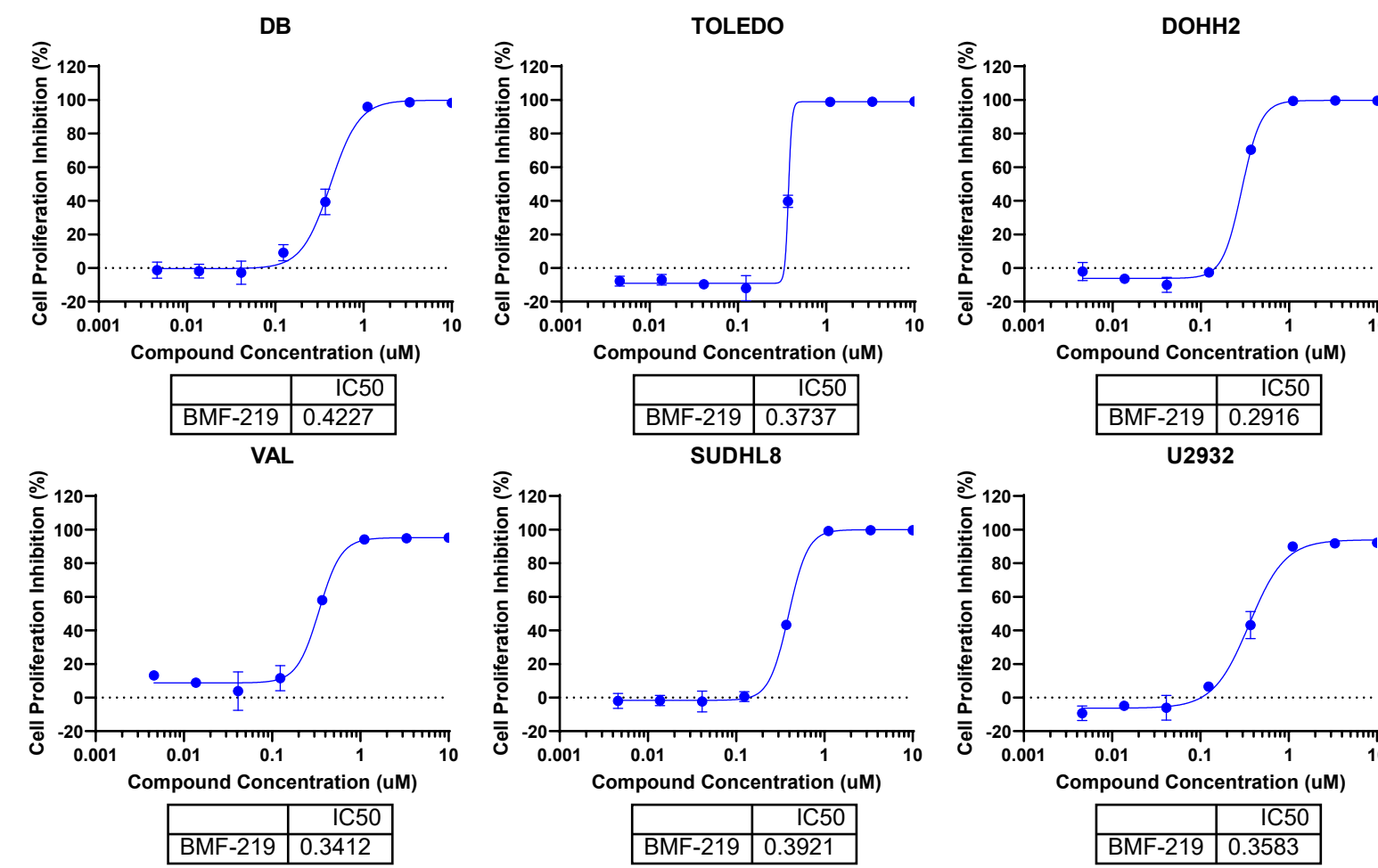
- AML and DLBCL cell lines were cultured in the presence of BMF-219 or clinical reversible menin inhibitors for 14 hours. Menin protein expression was measured by the Wes system and analyzed using the Compass software (automated western blotting, Protein Simple). Signal was normalized to GAPDH and referenced to DMSO control.
- DLBCL and MM 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 DLBCL PDX models and MM patient derived BMMCs 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.

RESULTS



BMF-219 reduces menin protein in DLBCL cells. Quantitation of menin protein expression in TOLEDO (DLBCL, DHL) cell line treated with BMF-219, clinical reversible menin inhibitors or preclinical reversible menin inhibitor, MI-503, for 14 hours.

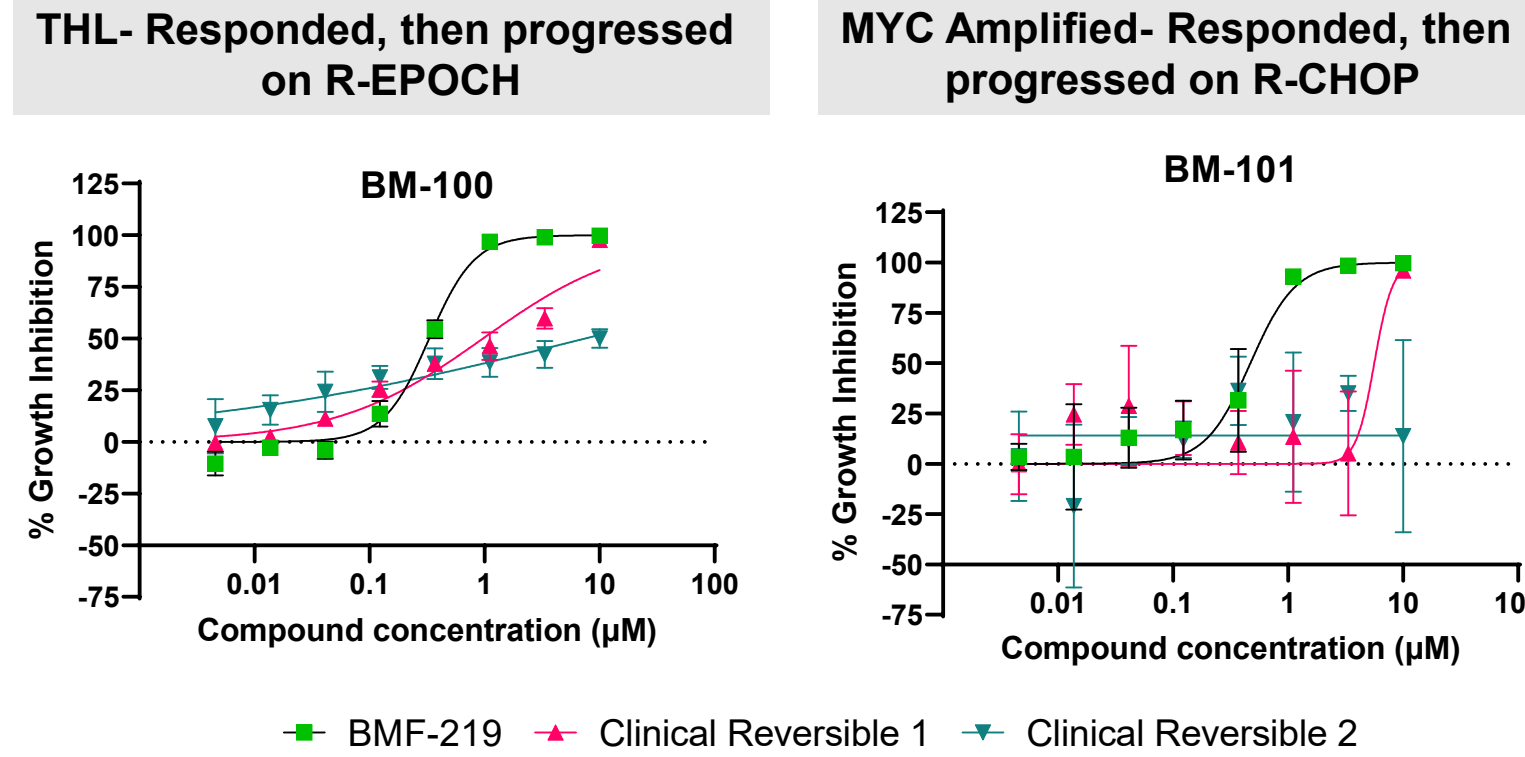
BMF-219 exerts potent cell lethality in DLBCL cell lines



DLBCL Cell Line	Category	Translocation	Average % Max Inhibition by BMF-219	Average IC ₅₀ ± Standard Deviation (µM)
DB	DHL	MYC/BCL2	98.5	0.407 ± 0.067
Toledo	DHL	MYC/BCL2	98.8	0.311 ± 0.065
DOHH2	DHL	MYC/BCL2	99.7	0.323 ± 0.031
VAL	THL	MYC/BCL2/BCL6	97.1	0.271 ± 0.070
U2932	DEL-ABC	MYC/BCL2 Overexpression	92.4	0.370 ± 0.012
SUDHL8	GCB	-	99.6	0.601 ± 0.209
Pfeiffer	GCB	-	99.6	0.167 ± 0.040
OCI-LY7	GCB	-	99.6	0.650 ± 0.260

Potent killing activity of BMF-219 at clinically relevant concentrations in representative DLBCL cell lines. Cell lines from DHL (DB, Toledo, DOHH2), THL (VAL), DEL (U2932), and GCB (SUDHL8) subtypes were cultured in the presence of BMF-219 at 8 dose concentrations ranging from 0.005 µM to 10 µM for 4 days and cell killing measured by Cell Titer Glo. Representative dose response curves are shown on the top. Average IC₅₀ values of at least two experiments, maximal percentage cell killing and cytogenetic background of DLBCL cell lines are summarized in the table.

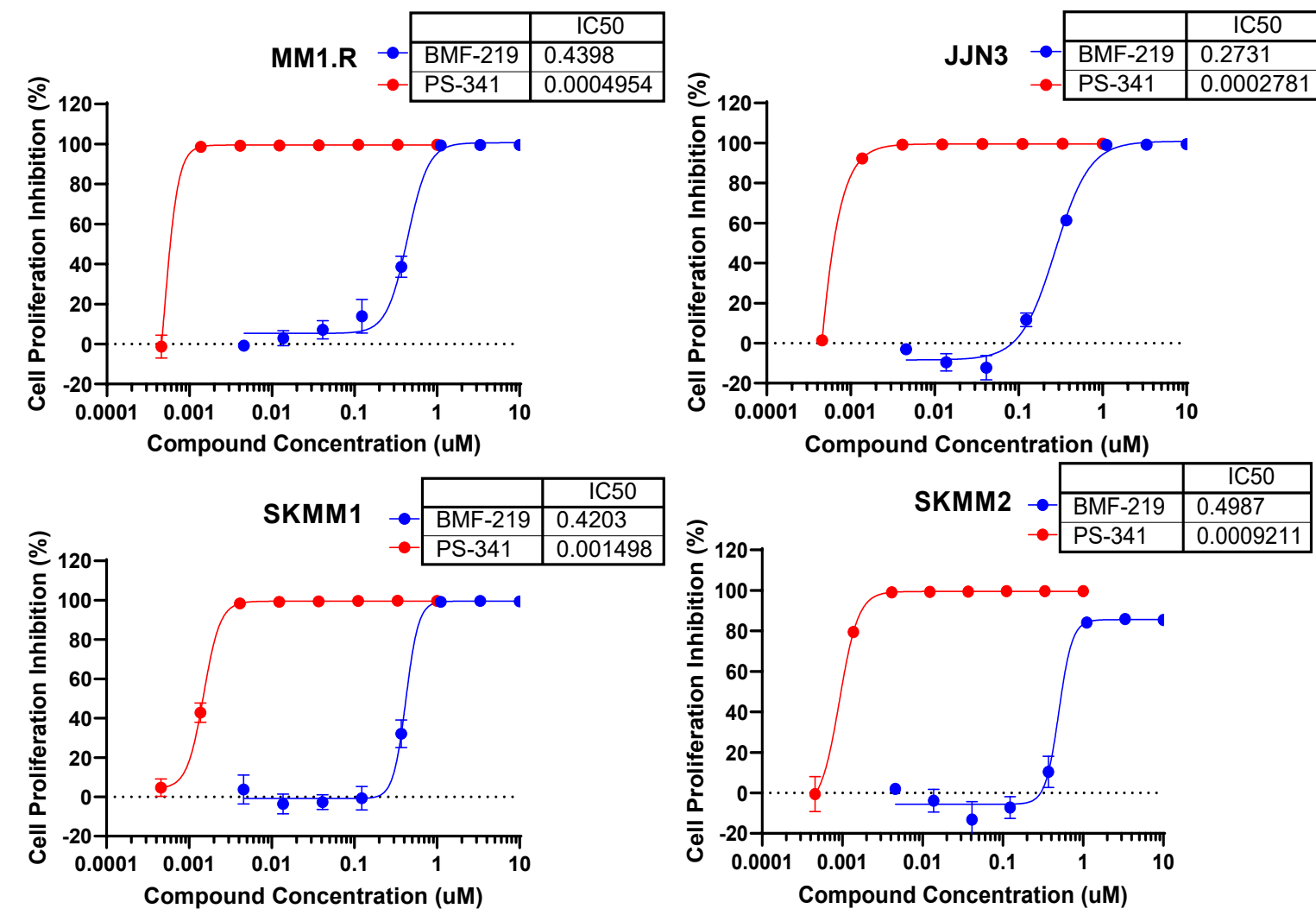
BMF-219 exerts pronounced lethality in DLBCL PDX models *ex vivo*



Treatment	Growth Inhibition IC ₅₀ (µM)	
	BM100	BM101
BMF-219	0.250	0.151
Clinical Reversible-1	0.969	5.63
Clinical Reversible-2	6.31	Max killing <30%

Growth inhibition of patient-derived DLBCL Triple Hit Lymphoma (THL) and MYC-amplified PDX samples treated with BMF-219 or clinical reversible menin inhibitors after 6 days of treatment.

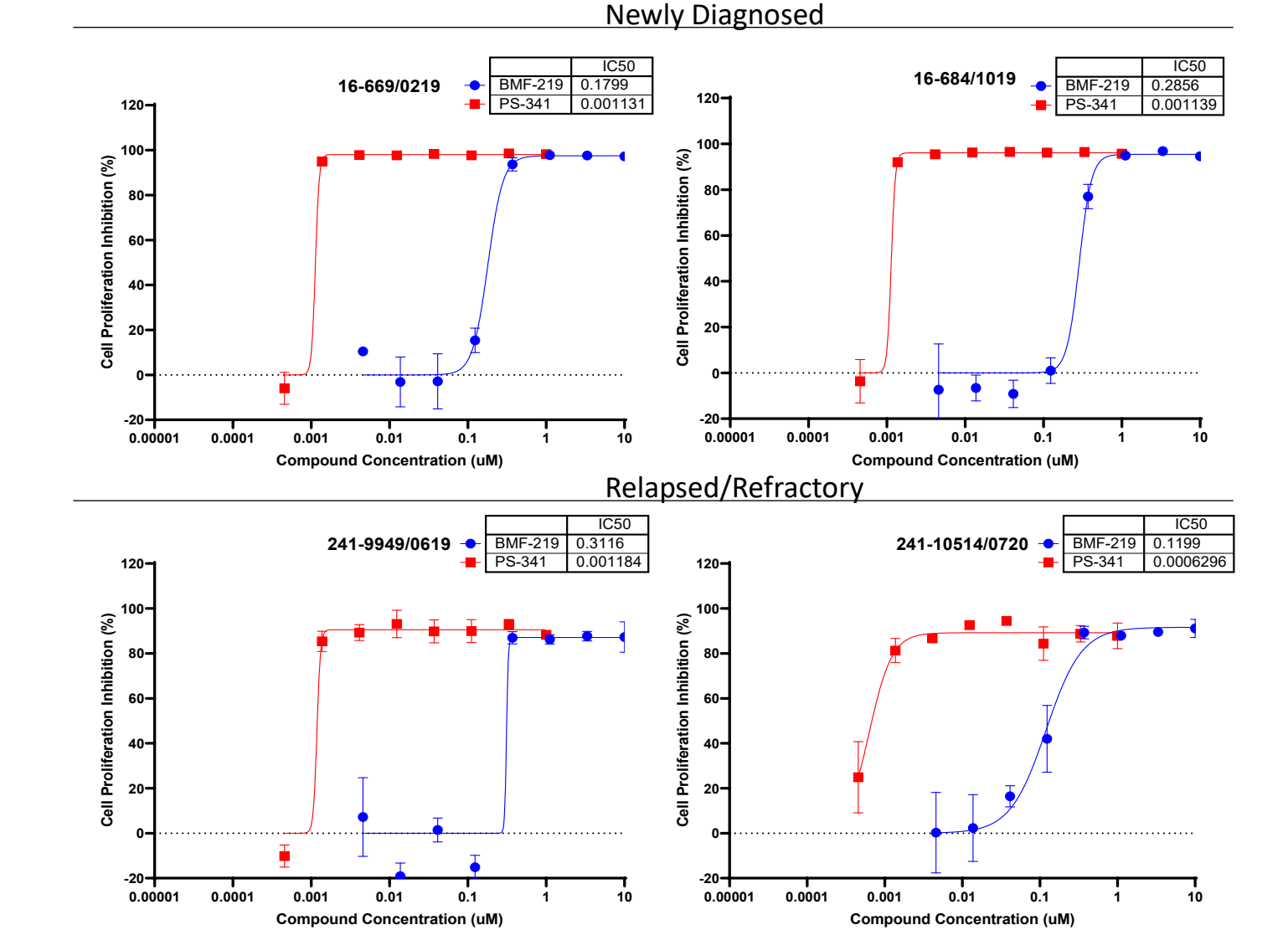
BMF-219 exerts >99% lethality against MM cell lines



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	0.467 ± 0.17
MM1.R	t(14;16)	KRAS G12A	99.6	0.462 ± 0.17
SKMM1	t(14;20)	NRAS G12A	99.2	0.467 ± 0.05
SKMM2	t(11;14)	TP53	80.2	0.654 ± 0.15
JN3	t(14;16)	NRAS Q61K	99.2	0.289 ± 0.02

Potent killing activity of BMF-219 at clinically relevant concentrations in representative MM cell lines. MM1.R, JN3, SKMM1 and SKMM2 cell lines were cultured in the presence of BMF-219 (blue) or PS-341 (red) at 8 dose concentrations ranging from 0.005 µM to 10 µM for four days and cell killing measured by Cell Titer Glo. Representative dose response curves are shown on the top. Average IC₅₀ values of at least two experiments, maximal percentage cell killing, and cytogenetic background of MM cell lines 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) High dose CPH (SC-mobilization) (responded) Consolidation (AutoSCT, double transplant) Bortezomib- maintenance (resistant) RVD #4 (resistant) PRD #4 (resistant)	p53 deletion-negative

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.

CONCLUSIONS

- BMF-219 exhibited high potency as a single agent against DHL, THL and DEL DLBCL cell lines, with IC₅₀ values of 0.27 µM and 0.37 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 achieved >99% cell lethality in MM cell lines with RAS mutations.
- BMF-219 demonstrated single-agent efficacy (IC₅₀ 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.

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