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Irreversible Menin Inhibitor, BMF-219, Exhibits Potent Cytotoxicity in KRAS-Mutated Solid Tumors

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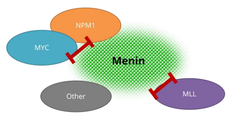
Introduction

- Kirsten rat sarcoma virus (KRAS) alterations are amongst the top oncogenic drivers and account for approximately one in seven of all human cancer. Within the US, KRAS mutations are most frequently found in high percentages of colorectal cancer (CRC), non-small cell NSCLC cancer (NSCLC) and pancreatic cancer.[1] These cancers respond poorly to standard-of-care agents, progress, and their management has been hindered by a lack of effective targeted therapies.
- BMF-219, is an orally bioavailable, selective irreversible inhibitor of menin, an important transcriptional regulator known to play a direct role in oncogenic signaling in multiple cancers.
- Preclinical data of BMF-219 show sustained potent abrogation of menin-dependent oncogenic signaling in vitro and in vivo.
- Results from Mia PaCa-2 cells prompted our exploration of the effects of BMF-219 in an expanded panel of KRAS-mutated solid tumors through in vitro, ex vivo PDX models, and KRAS-mutated gene expression.

Table 1. Estimated Target Population[1]

KRAS Type	Estimated Frequency of US Cases
CRC	~45%
NSCLC	~35%
Pancreatic	~90%



Mechanism of Action	Target Patient Population
MLL	MLL-r Acute Leukemia
NPM1	NPM1 mutant Acute Leukemia
MYC	DHT / DEL DLBCL
	Multiple Myeloma
	KRAS mutant (CRC, Lung, Pancreatic)
Other	Liquid and Solid Tumors

Methods

- Mia PaCa-2 cells were incubated with BMF-219 for 24 hours and analyzed by RNA-seq on the Illumina NextSeq 550 platform.
- BMF-219, clinical reversible menin inhibitor, or commercially available standard of care KRAS G12C inhibitor, sotorasib, were cultured with CRC, NSCLC, and pancreatic cancer cell lines for 4-days. Cell viability was measured using CellTiter Glo.
- Human ex vivo PDX tumor models harboring KRAS mutations were cultured with BMF-219 and clinical reversible menin inhibitors for 6-days. Cell viability was measured using CellTiter Glo.

Results

- Mia PaCa-2, a KRAS G12C mutated cell line, showed marked reduction of KRAS and MEN1 expression levels in MiaPaCa-2 cells at 0.5 µM and 1 µM after 24 hours BMF-219 treatment.
- An expanded panel of 14 CRC, NSCLC and pancreatic KRAS-mutated cell lines revealed single agent BMF-219 activity after a 4-day treatment. Most of the cell lines tested exhibited > 90% inhibition of growth, independent of KRAS mutation type. Sotorasib reached a maximum of 86-93% growth inhibition in three of ten G12C cell line. By contrast, BMF-219 inhibited cell viability ≥ 90% in seven of ten KRAS G12C NSCLC cancer lines.
- Human CRC, NSCLC and pancreatic ex vivo preclinical models with G12C and G12D KRAS mutations were all sensitive to BMF-219 6-day treatment. Complete abrogation of growth was observed in all samples with GI_{50} values ranging between 0.2 µM – 0.7 µM. Clinical reversible menin inhibitors were inactive in all preclinical models tested.

In Vitro Results

Figure 1. BMF-219 Cell-Killing in Mia PaCa-2 KRAS G12C cell line

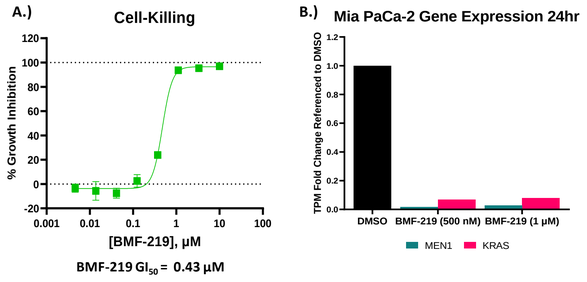


Figure 1. A.) BMF-219 induces cell killing of KRAS-mutated G12C cell line Mia PaCa-2 (Pancreatic cancer). Cells were treated with BMF-219 for 4 days and cell killing measured by Cell Titer Glo. Representative dose response curve is shown. GI_{50} is averaged from 2 independent experiments. **B.) BMF-219 treatment induced changes in KRAS and MEN1 in the KRAS-mutated cell line.** Each bar in the figure represents the Transcripts Per Million (TPM) normalized expression of MEN1 or KRAS genes colored by gene. Bar plots display the distribution of data referenced to the respective cell line DMSO controls.

Figure 2. Growth Inhibition of In Vitro KRAS Mutant Cell Lines

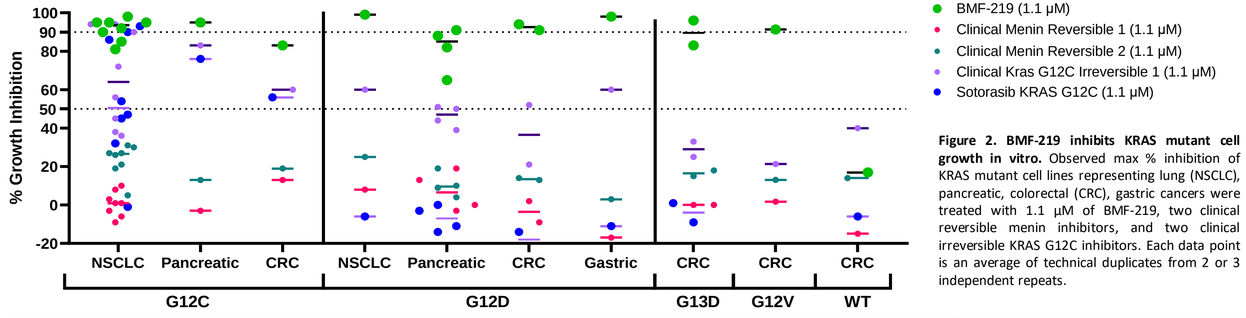


Figure 2. BMF-219 inhibits KRAS mutant cell growth in vitro. Observed max % inhibition of KRAS mutant cell lines representing lung (NSCLC), pancreatic, colorectal (CRC), gastric cancers were treated with 1.1 µM of BMF-219, two clinical reversible menin inhibitors, and two clinical irreversible KRAS G12C inhibitors. Each data point is an average of technical duplicates from 2 or 3 independent repeats.

Table 2. KRAS Mutant Cell Line GI_{50} Summary	KRAS G12C GI_{50} (µM)			KRAS G12D GI_{50} (µM)				KRAS G13D GI_{50} (µM)	KRAS G12V GI_{50} (µM)	WT GI_{50} (µM)
Tumor Type Type	NSCLC (8)	Pancreatic (1)	CRC (1)	NSCLC (1)	Pancreatic (4)	CRC (2)	Gastric (1)	CRC (2)	CRC (1)	CRC (1)
BMF-219	0.36 - 0.57	0.43	0.57	0.29	0.43-0.83	0.42	0.34	0.37-0.57	0.45	1.6
Clinical Menin Reversible 1	1.8 - 6.2, LR	3.1	6.9	2.0	4.1-7.2	6.5	3.8	3.9-6.3	3.2	LR
Clinical Menin Reversible 2	LR	LR	LR	LR	LR	LR	LR	LR	LR	3.3
Clinical KRAS G12C Irreversible	0.026 - 4.7	0.0038	0.017	0.84	1.1-4.0	1.1	0.70	1.4-2.3	1.8	1.3
Sotorasib KRAS G12C	0.033 - 5.6, LR	0.0054	0.035	LR	6.7, LR	LR	LR	LR	LR	LR

Limited Response (LR) represents $GI_{50} \geq 10$ µM. Values next to tumor cell type (#) are numbers of unique cell lines tested. Data represents mean of 2 or 3 independent repeats.

Ex Vivo Results

Figure 3.

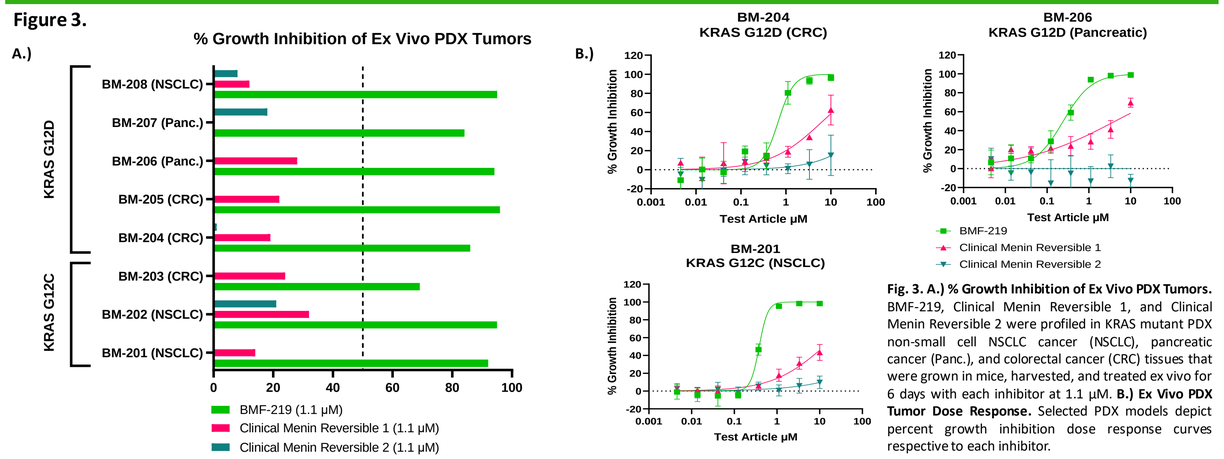


Fig. 3. A.) % Growth Inhibition of Ex Vivo PDX Tumors. BMF-219, Clinical Menin Reversible 1, and Clinical Menin Reversible 2 were profiled in KRAS mutant PDX non-small cell NSCLC cancer (NSCLC), pancreatic cancer (Panc.), and colorectal cancer (CRC) tissues that were grown in mice, harvested, and treated ex vivo for 6 days with each inhibitor at 1.1 µM. **B.) Ex Vivo PDX Tumor Dose Response.** Selected PDX models depict percent growth inhibition dose response curves respective to each inhibitor.

Table 3. KRAS Mutated PDX Specimen Profiles				GI_{50} Summary (µM)		
Patient Clinical Stage at Collection	Prior Therapy	KRAS Mutation	Specimen Type	BMF-219	Clinical Menin Reversible 1	Clinical Menin Reversible 2
BM-207 (Not Available)	N/A	G12D	Pancreatic	0.559	LR	LR
BM-206 (Not Available)	N/A	G12D	Pancreatic	0.244	4.27	LR
BM-204 (Stage IV)	1) 5-FU/Oxaliplatin; 2) 5-FU/Irinotecan/Bevacizumab; 3) 5-FU/Panitumumab	G12D	CRC	0.671	6.32	LR
BM-205 (Stage IV)	1) 5-FU/Oxaliplatin/Bevacizumab (mixed response) 2) Capecitabine/Irinotecan/Bevacizumab 3) Irinotecan/Cetuximab/Capecitabine (responded, progression unknown)	G12D	CRC	0.298	9.98	LR
BM-203 (Stage IV)	1) 5-FU/Oxaliplatin	G12C	CRC	0.624	8.23	LR
BM-208 (Stage IV)	1) Cisplatin/Etoposide (mixed response) 2) Carboplatin/Pemetrexed (mixed response) 3) Ramucirumab/Docetaxel (mixed response)	G12D	NSCLS	0.480	7.39	LR
BM-201 (Stage III)	1) Carboplatin/Nab-paclitaxel; 2) Carboplatin/Docetaxel	G12C	NSCLS	0.384	LR	LR
BM-202 (Not Available)	1) Cisplatin/Bevacizumab	G12C	NSCLS	0.352	7.75	LR

Conclusion

- Single agent treatment of BMF-219 in vitro at 1 µM in KRAS-mutated cell lines demonstrated higher cell killing in comparison to commercially available standard of care KRAS G12C inhibitor, sotorasib, and clinical KRAS G12C Irreversible.
- MEN1 and KRAS gene expression dramatically decreased after 24-hour treatment with BMF-219 in KRAS-mutated G12C cell line Mia PaCa-2 (Pancreatic cancer).
- BMF-219 achieves high cell killing broadly across KRAS-mutated ex vivo treated patient derived CRC, NSCLC, and pancreatic models.
- In comparison to two highly specific KRAS G12C inhibitors, BMF-219 exhibited potency broadly across KRAS-mutated cell lines and PDX tumor models indicating BMF-219 may provide therapeutic advantages over these KRAS mutation-specific inhibitors for use in CRC, NSCLC, and pancreatic cancers.
- At clinically relevant concentrations, potent clinical KRAS inhibitors like sotorasib are highly efficacious up to 50 % inhibition. However, at these concentrations BMF-219 is more proficient, achieving higher percent of cell killing, suggests that BMF-219 produces an increase in the depth of response.

References

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