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BMF-500: An Orally Bioavailable Covalent Inhibitor of FLT3 with High Selectivity and Potent Antileukemic Activity in FLT3-Mutated AML

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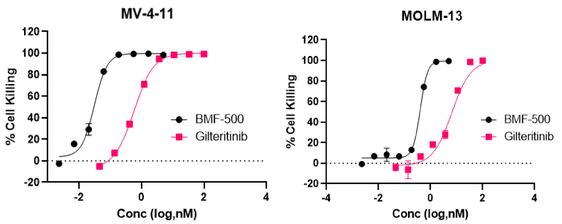
Introduction

- Fms-like tyrosine kinase 3 (FLT3), a member of the receptor tyrosine kinase family, is overexpressed on AML blasts and widely expressed in hematopoietic progenitor cells (Carow et al., 1996).
- Activating mutations of FLT3 are the most frequent genetic alterations in AML leading to FLT3 ligand-independent signaling and cellular proliferation, which account for approximately 30% of newly diagnosed adult AML patients and are associated with poor prognosis (Papaemmanuil et al., 2016).
- Although, several FLT3 inhibitors have entered clinical trials and reached commercialization; however, phospho-FLT3 target duration coverage, adverse events and dose-limiting toxicities often restrict the therapeutic window and limit their long-term use and efficacy (Kennedy VE & Smith CC, 2020).
- BMF-500 is a novel orally bioavailable, highly potent and selective covalent small molecule inhibitor of FLT3 with best-in-class potential, given its efficacy, durability, and selectivity in comparison to an existing FLT3 inhibitor.

Potent Target Inhibition

Figure 1.

A. Potent Cell-Based Activity in AML Cell Lines with FLT3 Mutations



Compound ID	MV-4-11 IC_{50} (nM)	MOLM-13 IC_{50} (nM)
BMF-500	0.03	0.30
Gilteritinib	1.7	6.5

Potent Coverage of FLT3 Inhibitor Resistance Mutations

B. NanoBRET Target Engagement Assay, IC_{50} (nM)

Cmpd ID	FLT3 WT	FLT3 (D835H)	FLT3 (D835V)	FLT3 (D835Y)
BMF-500	0.31	0.18	0.22	0.25
Gilteritinib	23.4	1.45	1.1	1.4

C. FLT3 Inhibitor Resistance Mutations Coverage, IC_{50} (nM)

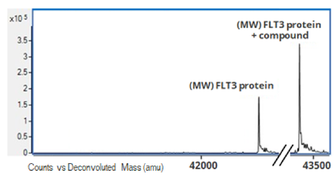
Cmpd ID	FLT3-ITD	FLT3-ITD-D835Y	FLT3-ITD-F691L
BMF-500	2 nM	5 nM	7 nM
Gilteritinib	7 nM	19 nM	98 nM

Figure 1. A.) Potent Cell-Based Activity in AML Cell Lines with FLT3 Mutations. Potency of BMF-500 and gilteritinib against FLT3 (WT) and FLT3-ITD enzyme-based biochemical assay (left) and cell-based 4-day CTG readout using MV-4-11 and MOLM-13 (right) cell lines. Representative dose response curves are shown for MV4-11 and MOLM-13 cell killing. **B.) NanoBRET Target Engagement Assay, IC_{50} (nM). C.) FLT3 Inhibitor Resistance Mutations Coverage, IC_{50} (nM).** Transformed cells expressing wild-type (WT), FLT3 D835H/V/Y variants, FLT-ITD, FLT-ITD-D835Y, and FLT3-ITD-F691L mutations were profiled via NanoBRET target engagement and Ba/F3 cell-based assays, respectively.

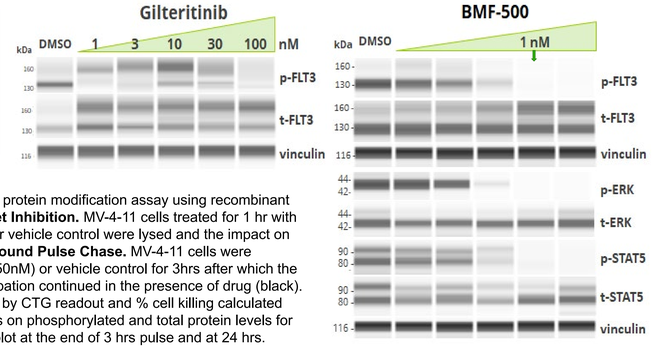
Potent and Durable Target Inhibition Leading to Effective Cell Killing

Figure 2.

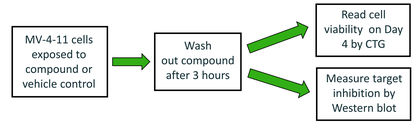
A. Protein Modification of FLT3

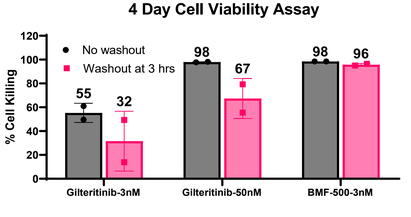


B. Target Inhibition



C. Compound Pulse-Chase Study





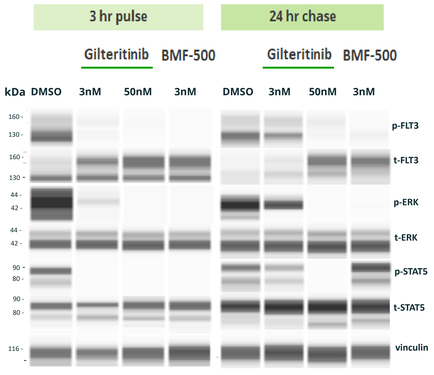
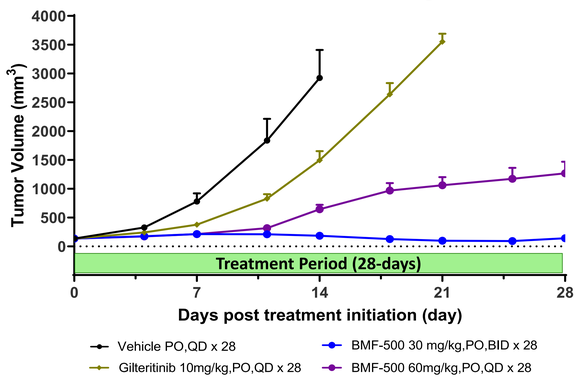


Figure 2. A) Protein Modification of FLT3. LC/MS protein modification assay using recombinant FLT3 protein in the presence of BMF-500. **B) Target Inhibition.** MV-4-11 cells treated for 1 hr with serial dilutions of BMF-500 (right), Gilteritinib (left) or vehicle control were lysed and the impact on FLT3 phosphorylation analyzed by WES. **C) Compound Pulse Chase.** MV-4-11 cells were treated with BMF-500 (3nM), Gilteritinib (3 nM and 50nM) or vehicle control for 3hrs after which the compound was either washed out (pink) or the incubation continued in the presence of drug (black). Viable cell count was measured at the end of Day 4 by CTG readout and % cell killing calculated relative to vehicle-treated control. In addition, effects on phosphorylated and total protein levels for FLT3, ERK and STAT5 were measured by immunoblot at the end of 3 hrs pulse and at 24 hrs.

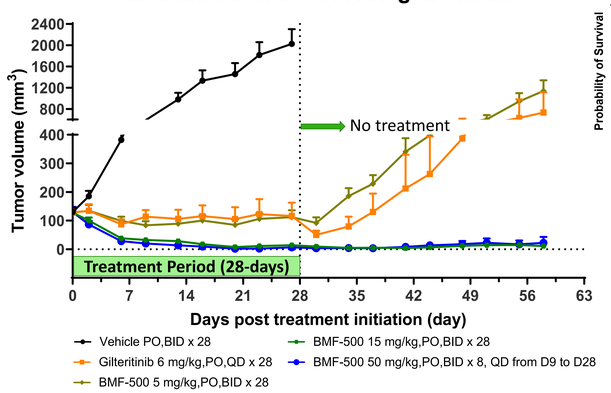
In Vivo Efficacy in Mouse Xenograft Models

Figure 3.

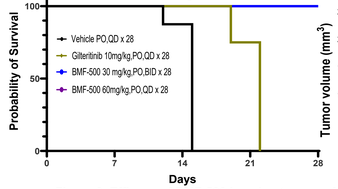
A. Subcutaneous MOLM-13 Xenograft Model



B. Subcutaneous MV-4-11 Xenograft Model



C. Overall Survival MOLM-13 Xenograft Model



D. Individual Tumor Volume MV-4-11 Xenograft Model

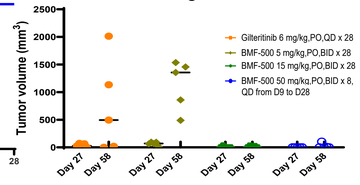


Figure 3. Efficacy of BMF-500 in subcutaneous AML xenograft models. Female nude mice bearing MOLM-13 (A), or MV-4-11 (B), xenograft tumors were dosed with BMF-500, gilteritinib or vehicle control for 28 days, and tumor volume was measured through the dosing phase and beyond. Vertical dotted line indicates termination of dosing (day 28). Data points represent group mean tumor volume. Error bars represent standard error of the mean (SEM). **C.) Overall Survival MOLM-13 Xenograft Model.** Kaplan-Meier probability of survival of female nude mice bearing MOLM-13 xenograft. **D.) Individual Tumor Volume MV-4-11 Xenograft Model.** Individual tumor volume trace of female nude mice bearing MV-4-11 xenograft model.

High Selectivity

Figure 4.

A. Kinase Profile

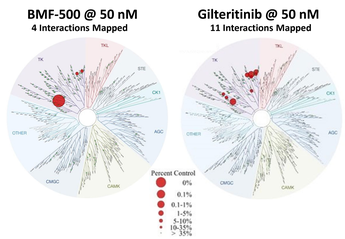


Figure 4A. Kinase Profile. Map of the human kinome is shown for 169 kinases for BMF-500 (left) and gilteritinib (right) as profiled against a panel of 169 kinases at 50 nM top concentration (>100x target IC_{50} of BMF-500). The red circles denote inhibition of activity greater with size scale as shown in legend.

B. 5-Day Cytotoxicity Profile

Cell Line	Tumor Type	BMF-500	Gilteritinib
SW684	Fibrosarcoma	>1	>1
A549	NSCLC	>1	0.278
BV-173	Leukemia (CML)	>1	0.740
CGTH-W-1	Carcinoma, metastatic	>1	0.455
Daudi	Burkitt's lymphoma	>1	>1
HCT-116	Carcinoma	>1	>1
Jurkat	Acute T-cell leukemia	>1	0.947
HL-60	Leukemia, acute promyelocytic	>1	0.445
LS411N	Carcinoma, Duke's type B	>1	>1
MOLT-4	Leukemia (ALL)	>1	>1
MCF7	Adenocarcinoma	>1	>1
MV-4-11	Leukemia (acute myelomonocytic	**<0.001**	**0.003**
RS4;11	Leukemia (acute lymphoblastic)	>1	0.233
SaOS2	Osteosarcoma	>1	0.236
SK-N-AS	Neuroblastoma	>1	>1
SKOV3	Adenocarcinoma	>1	0.804
Thp1	Leukemia (acute monocytic)	>1	>1
WiDr	Colorectal adenocarcinoma	>1	0.268
CCRFCEM	Leukemia (acute lymphoblastic)	>1	>1
RL95-2	Carcinoma	>1	0.868

Figure 4B. 5-Day Cytotoxicity Profile. BMF-500 and gilteritinib were profiled against a panel of 20 cancer cell lines in a 5-day cell viability assay, using CTG readout. Top concentration tested was 1 µM. IC_{50} values are listed. Cell lines represent different tumor tissue types as indicated. MV-4-11 was the only cell line in the panel known to harbor a FLT3 activating mutation (FLT3-ITD).

Conclusion

- The covalent small molecule inhibitor BMF-500 exhibits potent inhibition of FLT3 receptor kinase and excellent cell killing in FLT3-ITD AML cell lines.
- Transformed cells expressing key FLT3 inhibitor resistance variants, including D835Y/V/H activation loop and F691L gatekeeper point mutations show high sensitivity to BMF-500, highlighting a clear differentiation to gilteritinib.
- Covalent modification of FLT3 by BMF-500 leads to constitutive on-target inhibition of phospho-FLT3 and its down-stream signaling in a dose dependent manner in the target cell.
- In MV-4-11 cells, 3-hour exposure followed by washout of BMF-500 was sufficient to produce longer duration of response and greater cell killing that outperformed continuous exposure of gilteritinib at 3 nM.
- BMF-500 demonstrates antitumor activity with sustained tumor regression, improved survival and is well tolerated with body weight maintenance across treatment groups in two preclinical mouse xenograft models.
- The kinase profile of BMF-500 revealed high target selectivity and selective cytotoxicity profile against a panel of non-target cancer cell lines suggesting minimal off target liabilities.

References

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