Abstract 4939 AACR April 14-19, 2023

Combinatorial approach using covalent menin inhibitor, BMF-219, and/or covalent FLT3 inhibitor, BMF-500, with MEK or BCL2 blockade potentiates therapeutic use in AML

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Introduction

- Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease characterized by a highly diverse genomic landscape.
- Despite recent advances in therapies, treatment outcome remains variable and largely defined by genomic abnormalities such as gene fusions, copy number alterations and point mutations.
- Mutations in epigenic modifiers, nucleophosmin (NPM1c), signaling and kinase pathway such as KMT2A-re-arrangements (KMT2A-r), internal tandem duplication (ITD) insertions in FLT3, and NRAS mutations are amongst the highest alterations in AML patients with a propensity towards poor response to treatment and overall disease outcome.
- Additionally, the use of FLT3 as monotherapy can confer drug-resistance and relapse, limiting the ability to achieve long-lasting response to treatment.
- Combinatorial strategies would be required to combat resistance and maximize duration of antitumor activity.
- Combination of menin and/or FLT3 inhibitors with BCL2 and MEK inhibitors may offer the potential to achieve increased antitumor activity and overcome AML resistance.
- Here we explored the use of our clinical-stage covalent menin inhibitor, BMF-219, and BMF-500, a covalent FLT3 inhibitor, in combination with each other and in combination with BCL2 and MEK inhibitors in MLLr and FLT3-mutated acute leukemia cell lines, MV-4-11 and MOLM-13. MOLM-13 (MLL-AF9 fusion) represents 24% of AML MLL patient population while MV-4-11 (MLL-AF4 fusion) represents 2% of AML MLL patient population.

BMF-219 down regulates FLT3 transcript

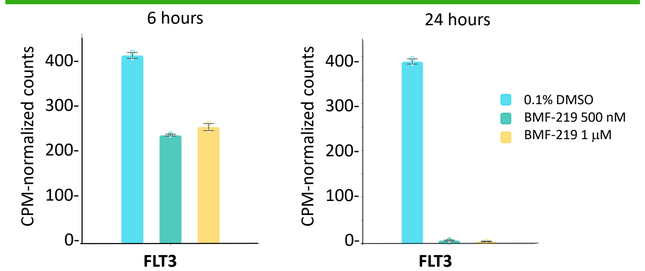


Figure 1. FLT3 expression data in MOLM-13 cells treated with BMF-219 after 6 and 24 hrs. mRNA expressions were calculated using the cpm function in the edgeR R package with log=F. Each bar represents two replicate samples. Housekeeping gene, *GAPDH*, was not affected by BMF-219 treatment.

Methods

- AML cells were cultured in the presence of BMF-219, BMF-500, trametinib or venetaclax as single agents or combinations and the antileukemic activity was assessed on Day 4. Cell viability was measured by CellTiterGlo.
- RNA-seq was conducted 6 and 24 hours after BMF-219 treated on the Illumina Novoseq 500 platform and analysis was performed using Pluto (https://pluto.bio). Differential gene expression was determined using the edgeR function.
- ZIP synergy scores were generated with SynergyFinder R package.

BMF-219 and BMF-500 in combination induced higher cell killing at lower single agent concentrations

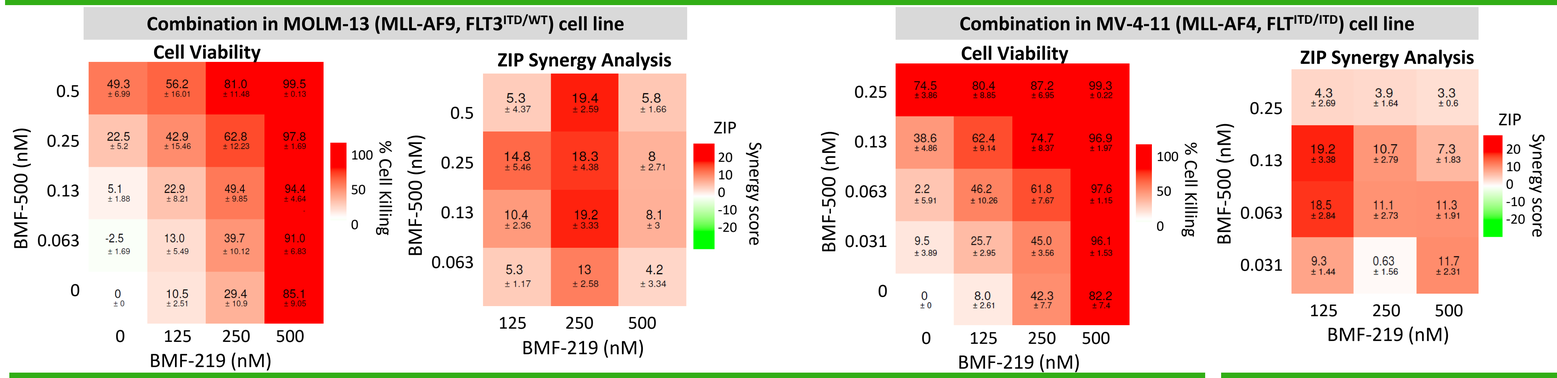
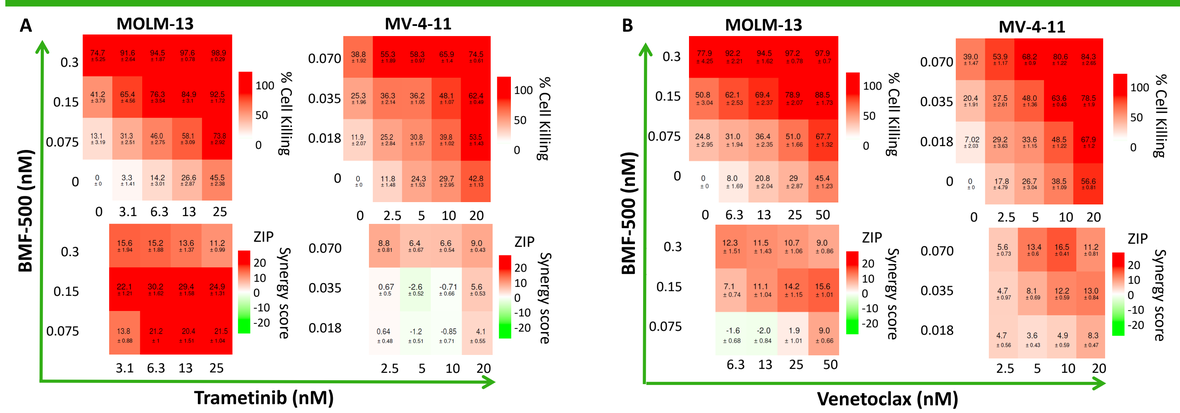


Figure 2. Combination treatment of AML cells with BMF-219 and BMF-500. MOLM-13 (A) and MV-4-11 (B) cells were treated with BMF-219 and BMF-500 as single agents or in combination. Viability was measured by CellTiterGlo on Day 4. Percent inhibition (±SEM) of cell viability was averaged from 6 independent repeats. SEM = standard error of mean. ZIP synergy scores were generated with SynergyFinder R package.

Below -10 = Antagonistic
-10 to +10 = Additive
Above +10 = Synergistic

BMF compounds combined with MEK or BCL2 inhibitors elicit additivity



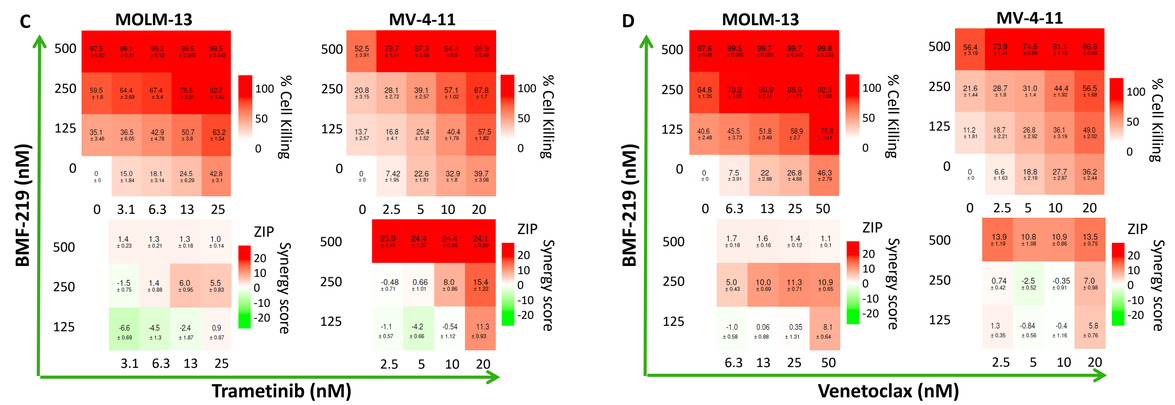


Figure 3. Combination treatment of BMF-219 or BMF-500 in MOLM-13 and MV-4-11 cells. Cells were treated with BMF-500 in combination with trametinib (A) or venetoclax (B), or BMF-219 in combination with trametinib (C) or venetoclax (D) for 4 days. Results were averaged from 3 independent studies. Assay and analysis details are the same as in Figure 2.

Below -10 = Antagonistic
-10 to +10 = Additive
Above +10 = Synergistic

Conclusions

- MOLM-13 represents AML with FLT3 ITD heterozygous and MLL-AF9 fusion, while MV-4-11 represents AML with homozygous FLT3 ITD and MLL-AF4 fusion.
- BMF-219 down regulates FLT3 expression as early as 6 hours and by 24hr expression is inhibited by more than 90%.
- BMF-219 and BMF-500 in combination shows beneficial effects affording higher cell killing at lower concentrations.
- Increased cell killing is achieved when BMF-219 is combined with the MEK inhibitor, trametinib, or with the BCL2 inhibitor, venetoclax, at specific concentrations, especially in MV-4-11 (MLL-AF4 fusion, 2% MLL patients).
- BMF-500 combined with the MEK inhibitor, trametinib achieved more than additive cell killing in the MOLM-13 cells vs MV4-11.
- BMF-500 combined with the BCL2 inhibitor, venetoclax achieved additive cell killing in both cell lines.
- Collectively, our results demonstrate the utility of combination approaches of menin and FLT3 covalent inhibitors with MEK and BCL2 blockade to achieve higher antileukemic cell killing with lower drug concentrations.
- These data provide initial pre-clinical rationale for combining pathway specific inhibitors as a promising therapeutic strategy for further investigation in acute leukemia

References

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2. Yadav, B.; Wennerberg, K.; Aittokallio, T.; Tang, J. Searching for Drug Synergy in Complex Dose-Response Landscapes Using an Interaction Potency Model. Comput Struct Biotechnol J 2015, 13, 504–513. doi:10.1016/j.csbj.2015.09.001

Acknowledgement: Thank you Brian Munneke for ZIP synergy score batch analysis.