4172: Efficacy of Belvarafenib with and without Cobimetinib in Preclinical Models of Ras Pathway-Mutant AML

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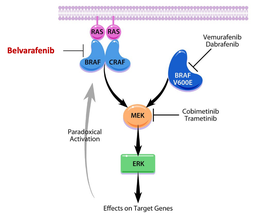
INTRODUCTION

Outcomes for children with acute myeloid leukemia (AML) remain poor, with ~40% dying from refractory leukemia or treatment-related toxicity (Gamis et al. 2014). NRAS, KRAS, and NF1 mutations occur in over 40% of pediatric AMLs (Bolouri et al. 2018), but efforts to therapeutically target the RAS/mitogen activated protein kinase (MAPK) pathway have been largely unsuccessful due, in part, to dose-limiting adverse side effects and reactivation of downstream molecules upon target inhibition. Rational combination therapies are a logical strategy for targeting hyperactive RAS/MAPK signaling in AML and other cancers.

Belvarafenib is a novel pan-Raf inhibitor with several important advantages over first-generation Raf inhibitors such as vemurafenib and dabrafenib that are are ineffective in cancers characterized by NRAS, KRAS, or NF1 mutations. Belvarafenib potently inhibits BRAF and CRAF hetero- and homo-dimers. Early phase clinical trials of belvarafenib in relapsed/refractory BRAF-mutant melanoma have demonstrated promising activity. (Yen et al., 2021). The goals of our study was to evaluate single agent activity and biochemical mechanism(s) of action in NRAS/KRAS-mutant AML cell lines and in preclinical models. Informed by previous studies in solid cancer cell lines (Yen et al., 2021), we also investigated belvarafenib in combination with cobimetinib, an FDA-approved allosteric MEK inhibitor.



METHODS

We used a panel of NRAS or KRAS mutant human AML cell lines. Viability was determined by CellTiter-Glo. Synergy was assessed by Bliss Independence and Chou Talalay methods. Transcriptome and proteomic profiling were performed (Pucciarelli et al. 2020). Mouse AMLs were generated using retroviral insertional mutagenesis (Li et al. 2011). Cryopreserved primary AML cells were injected intravenously into sublethally irradiated recipients and treated daily with vehicle, belvarafenib, cobimetinib, or the combination until disease progression. Survival curves were generated using Kaplan-Meier analysis

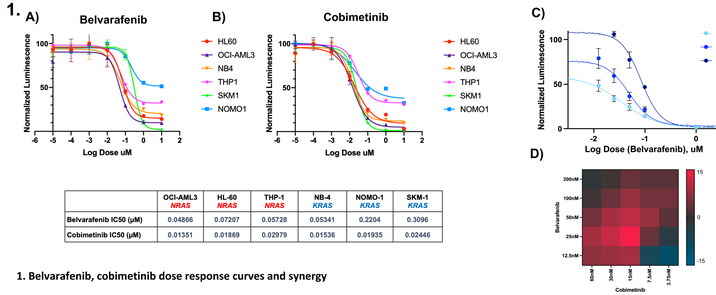
AIMS

1. To evaluate the efficacy of belvarafenib with and without cobimetinib in models characterized by hyperactive Ras signaling.

2. To characterize mechanisms of response and resistance to belvarafenib and the combination in these models.

RESULTS

1.

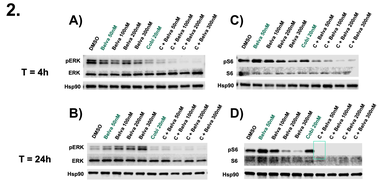


	OCI-AML3 *NRAS*	HL-60 *NRAS*	THP-1 *NRAS*	NB-4 *KRAS*	NOMO-1 *KRAS*	SKM-1 *KRAS*
Belvarafenib IC50 (μM)	0.04866	0.07207	0.05728	0.05341	0.2204	0.3096
Cobimetinib IC50 (μM)	0.01351	0.01869	0.02979	0.01536	0.01935	0.02446

1. Belvarafenib, cobimetinib dose response curves and synergy

Six AML cell lines (3 *NRAS* mutant and 3 *KRAS* mutant) were treated with belvarafenib (A) or cobimetinib (B) for 72 hours and proliferation was assessed via CellTiter-Glo (CTG). IC50 values are shown below. C) CTG dose response curve showing OCI-AML3 cells exposed to a combination of belvarafenib and cobimetinib. Synergy was assessed using BLISS Independece analysis with positive synergy scores in red (D).

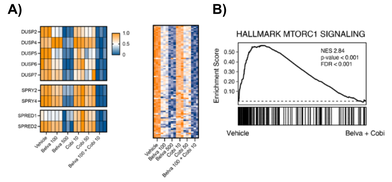
2.



2. Effects of belvarafenib and cobimetinib on the phosphorylation of Ras effector proteins in OCI-AML3 cells

A) OCI-AML3 cells were exposed to belvarafenib at several concentrations (50nM, 100nM, 200nM, and 300nM), cobimetinib 20nM, or the combination of cobimetinib plus belvarafenib at all doses, for 4 or 24 hours. Western blotting was performed for pERK/ERK and pS6/S6. A, B) Belvarafenib has less of an effect on pERK than cobimetinib at both time points, with a synergistic effect at both time points (more prominent at 4 hours). C, D) While low-dose belvarafenib and cobimetinib are largely ineffective at decreasing pS6 as single agents, the two agents have a strongly synergistic effect at 4 and 24 hours. Note the absence of any phosphorylated ERK at 24 hours with doses as low as belvarafenib 50nM plus cobimetinib 20nM.

3.



3. Heatmap and GSEA of RNA-seq data from OCI-AML3 cells exposed to belvarafenib and/or cobimetinib for 24h

Cells were treated for 24h with belvarafenib (100nM and 500nM) and cobimetinib (10nM and 50nM) alone and in a low-dose combination. A) Curated expression levels of genes involved in MAPK feedback that are expressed at significant levels in AML cells. Expression of negative regulators of the MAPK pathway (e.g. DUSPs, SPRY and SPRED), is decreased after treatment with the low-dose belvarafenib/cobimetinib combination or high-dose belvarafenib. B) Heatmaps and GSEA showing the effects of belvarafenib, cobimetinib, and combination treatment on mTORC1-associated gene sets.

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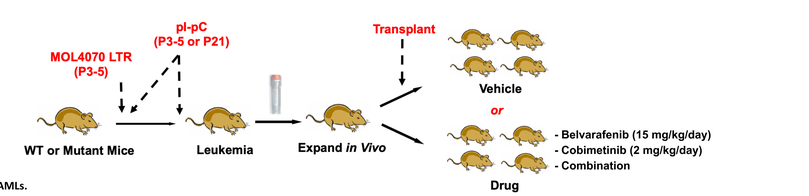
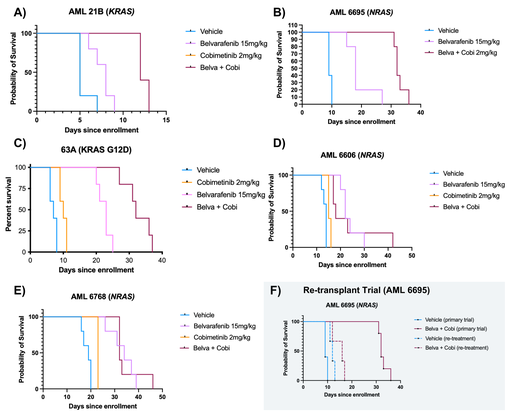


3. Preclinical trials in mouse AMLs.

Leukemias were initiated by injecting WT, Nras, or Kras mutant mice with MOL4070LTR. Early passage leukemia cells are cryopreserved and then expanded in "factory" mice. These cells are then transplanted into cohorts of congenic, immunocompetent recipient mice that receive either control vehicle or drug treatment. Mice are treated continuously until relapse, at which point leukemias are isolated for molecular and functional analyses.

4.



4. Preclinical trials using belvarafenib and cobimetinib in primary murine AMLs: Belvarafenib extends survival in 5/5; the addition of cobitinib extends survival further in 3/5.

A-E) Kaplan-Meier survival analysis of recipient mice that received vehicle (blue), cobimetinib (orange), belvarafenib (pink) or the combination (maroon). F) Leukemic cells from the longest surviving combination-treated mouse in AML 6695 (Fig. 5B) were used to re-transplant six additional recipients that were re-treated with either a vehicle or the belvarafenib/cobimetinib combination. This relapsed leukemia displayed phenotypic resistance, as indicated by the markedly reduced survival of the belvarafenib/cobimetinib re-treated mice, which was similar to the vehicle re-treated recipients.

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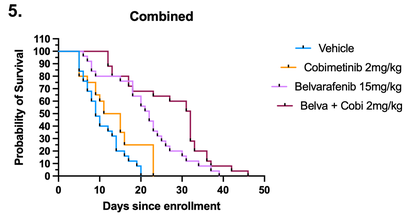

5. Combined Kaplan-Meier curve of all 5 distinct murine AML trials.

Kaplan-Meier survival analysis of recipient mice that received vehicle (blue), cobimetinib (orange), belvarafenib (pink) or the combination (maroon). Data were pooled from 5 independent trials: three NRAS-mutant AMLs (5B, D, E) and two KRAS-mutant AMLs (5A, C). $p < 0.05$ for the comparisons all curves to the vehicle curve.

CONCLUSIONS

Belvarafenib and cobimetinib demonstrated potent single agent and synergistic activity in six AML cell lines with NRAS or KRAS mutations. Biochemical analysis of downstream effector proteins in AML cell lines revealed differences in ERK phosphorylation levels and the activation states of other key Ras effector molecules in AML cells exposed to belvarafenib and cobimetinib. Transcriptome (RNA-seq) and biochemical analyses uncovered down-regulation of mTORC-regulated transcriptional programs and of key Akt/mTORC pathway proteins in response to belvarafenib, but not cobimetinib. In preclinical trials of primary Nras/Kras-mutant murine AMLs, belvarafenib significantly extended survival in five independent models, and was synergistic with cobimetinib in three of these. We observed resistance to belvarafenib and the belvafenib/cobimetinib combination in some in vivo models; whole-exome sequencing has identified several candidate resistance genes that are currently under investigation.

Altogether, these studies support evaluating belvarafenib as a single agent and in combination with cobimetinib in AMLs with NRAS or KRAS mutations.

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