Combined inhibition of farnesyltransferase and MEK is effective in HRAS-mutant rhabdomyosarcoma

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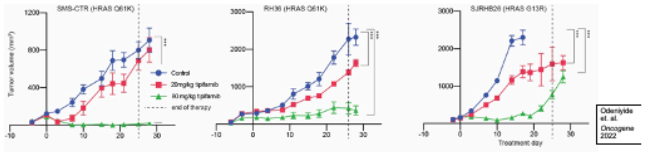
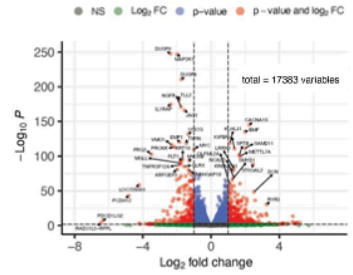
Background and Rationale

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of childhood, and RAS pathway mutations are the known driver mutations in the majority of fusion-negative (FN) RMS. Recent studies have demonstrated that HRAS mutations are enriched in infant cases of FN-RMS and can be associated with an aggressive clinical course and inferior outcomes. Using HRAS-mutant RMS cell lines and xenograft models, we have demonstrated that tipifarnib (farnesyl transferase inhibitor, FTI) decreases ERK signaling, decreases *in vitro* proliferation, and decreases *in vivo* tumor growth. The effects of tipifarnib can be incomplete, however, leading only to partial or short-lived responses. Limitations may be due to adaptive or acquired resistance, suggesting that HRAS-mutated FN-RMS may be sensitive to pathway inhibition with combination therapy that prevents or delays the emergence of adaptive resistance. Trametinib (MEKi) inhibits tumor growth in xenograft models of FN-RMS but has only modest activity as a single agent, potentially due to release of negative feedback and activation of upstream signaling. The efficacy of inhibition with FTI and MEKi have not been previously explored in RAS-driven FN-RMS.



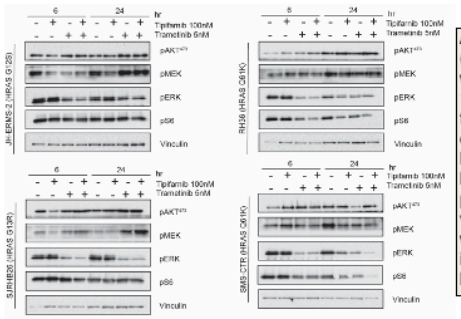
NSG mice bearing HRAS-mutant, NRAS-mutant, KRAS-mutant, and RAS WT xenografts were treated with vehicle, or tipifarnib at 20 or 80 mg/kg twice daily (5 days on/2 days off) for three weeks. The average tumor volume is graphed as a function of days on treatment. Error bars represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, unpaired Student t-test. Statistical comparisons are relative to control groups on treatment day 28.

Tipifarnib downregulates canonical ERK transcriptional targets



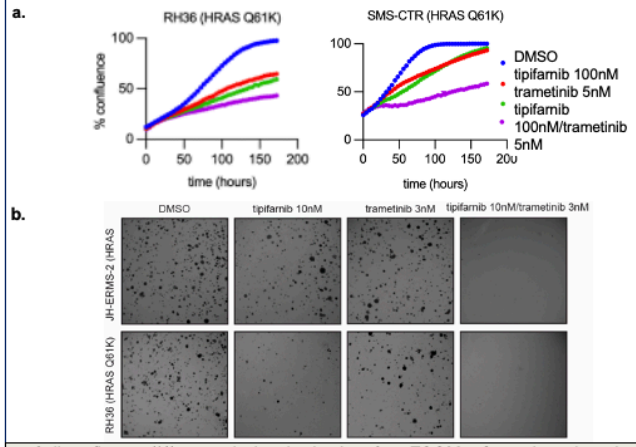
The R package EnhanceVolcano was used to generate volcano plots of the differentially expressed genes for SMS-CTR (HRAS Q61K) at 24 hrs

Trametinib potentiates the effects of tipifarnib on ERK signaling



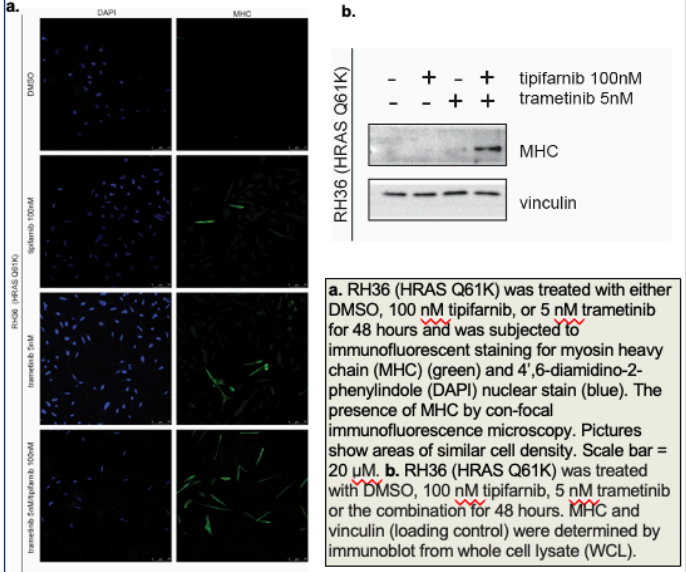
A panel of RMS cell lines (mutations as indicated) were treated with DMSO, 100 nM tipifarnib, 5 nM trametinib or the combination for 6 or 24 hours. Phospho- and total levels of ERK and PI3K pathway components, and vinculin (loading control) were determined by immunoblot from whole cell lysate (WCL).

Tipifarnib and trametinib more potently reduces HRAS-mutant cell proliferation



a. Cell confluency (%) was calculated using IncuCyte ZOOM software based on phase-contrast images of SMS-CTR and RH36 cells from 0 to 120 hours at DMSO, 100 nM tipifarnib, 5 nM trametinib or the combination. Each data point represents six wells. b. Representative images of RMS cell lines grown in soft agar and treated with either DMSO, 10 nM tipifarnib, 3 nM trametinib or the combination for three weeks.

Tipifarnib in combination with trametinib promotes myogenic differentiation



a. RH36 (HRAS Q61K) was treated with either DMSO, 100 nM tipifarnib, or 5 nM trametinib for 48 hours and was subjected to immunofluorescent staining for myosin heavy chain (MHC) (green) and 4',6-diamidino-2-phenylindole (DAPI) nuclear stain (blue). The presence of MHC by con-focal immunofluorescence microscopy. Pictures show areas of similar cell density. Scale bar = 20 μM. **b.** RH36 (HRAS Q61K) was treated with DMSO, 100 nM tipifarnib, 5 nM trametinib or the combination for 48 hours. MHC and vinculin (loading control) were determined by immunoblot from whole cell lysate (WCL).

Conclusions

Vertical RAS pathway inhibition with tipifarnib and trametinib inhibits 2D and 3D cell growth, ERK signaling and promotes MHC expression in HRAS mutated human RMS cell lines. These results suggest that the combination of tipifarnib and trametinib could be tested in a trial for patients with HRAS mutated RMS.

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