The combination of ulixertinib (ERK1/2 inhibitor) and *KRAS*^{G12C} inhibition demonstrates significant efficacy in preclinical models

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Abstract #2692

Background

- Ulixertinib (BVD-523) is a first-in-class and best-in-class small molecule inhibitor of ERK1/2 currently being investigated in oncology clinical trials, both as a single agent and in combination with other therapeutics.
- Ulixertinib has demonstrated efficacy in patients with tumors harboring alterations within the *RAS-MAPK* pathway[1].
- The *KRAS*^{G12C} mutation occurs in approximately 10% of non-small cell lung cancer (NSCLC), 3% of colorectal cancer, and 1-2% across all other tumor types[2].
- KRAS^{G12C} mutant-inhibitors, including AMG-510 (sotorasib), MRTX849 (adagrasib), and JDQ443 have demonstrated efficacy in *KRAS*^{G12C}-mutant cancers, including NSCLC.
- Clinically described mechanisms of acquired resistance to KRAS^{G12C} inhibitors converge on reactivation of the *RAS-MAPK* pathway (Figure 1)[3-6].
- We hypothesized combining ulixertinib with a KRAS^{G12C} inhibitor would circumvent resistance to single agent KRAS^{G12C} inhibition, generating increased magnitude and duration of response compared to either single agent alone.

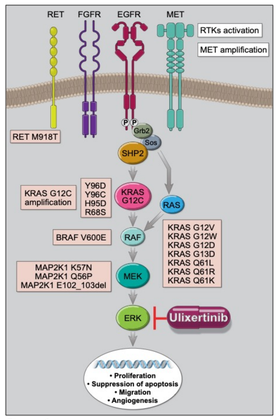
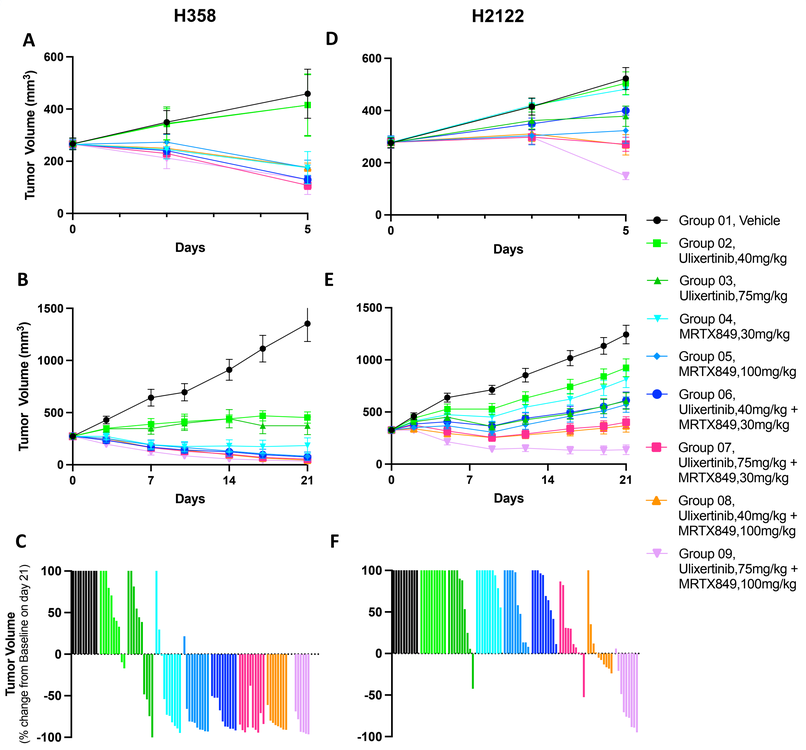


Figure 1

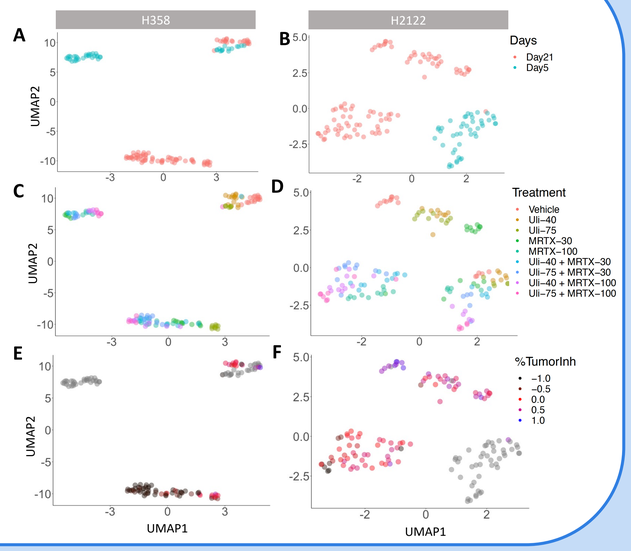
1. Ulixertinib in combination with *KRAS*^{G12C} inhibition is efficacious



- The efficacy of ERK1/2 inhibitor, ulixertinib (uli), in combination with KRAS^{G12C} inhibitor, adagrasib (MRTX849), was assessed in cell line-derived xenograft models harboring *KRAS*^{G12C} mutations. Models were selected based on response to single agent adagrasib, ranging from sensitive (H358) to partially responsive (H2122)[7].
- Combining uli with MRTX849 (MRTX) resulted in superior tumor growth inhibition compared to dosing of either single agent alone in both models. In the highly sensitive model, H358, high- and low-dose combination was efficacious. In H2122, the addition of low- or high-dose ulixertinib to 30mg MRTX849 resulted in equal or improved efficacy, respectively, compared to 100mg MRTX849 monotherapy. High-dose combination treatment (Groups 8 and 9) yielded robust regression compared to all other treatment groups.
- Tumor volume over time is shown for groups dosed to day 5 and day 21 for H359 (A, B) and H2122 (D, E). Tumor volume percent change from baseline at day 21 is shown for H359 (C) and H2122 (F). Visualization of growth is capped at 100% (C, F).

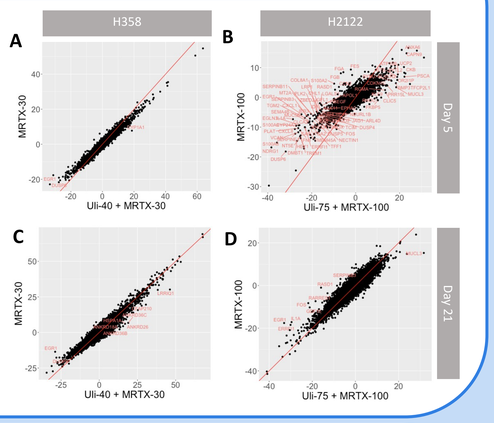
2 . Gene expression clusters by treatment and response

- RNA-sequencing was performed on H358 and H2122 tumors collected 2-hours post-dose on day 5 and day 21.
- Uniform Manifold Approximation and Projection (UMAP) was applied to the gene expression data to visualize patterns of clustering.
- In both models, H358 and H2122, tumors clustered by days on treatment (A, B), treatment groups (C, D) and percent tumor growth inhibition (E, F).
- Overall, these patterns provide a framework to elucidate impactful changes in gene expression with treatment response.

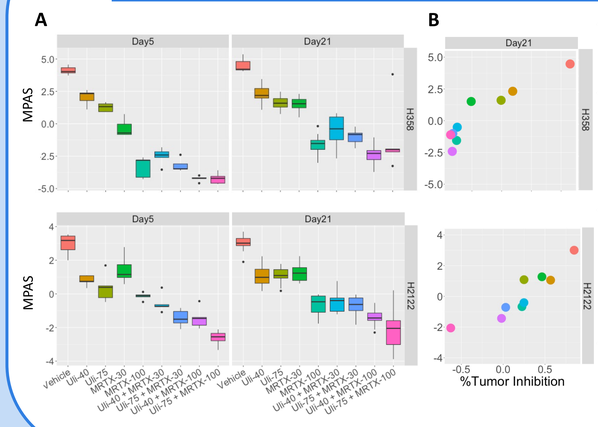


3. MRTX849 + uli combination engages components of MAPK signaling

- The H358 model is highly sensitive, demonstrating robust regressions for all uli + MRTX combinations. We hypothesized that the lower dose MRTX combination with uli, which shows synergy, might be clinically relevant with lower toxicity. Comparing the gene expression profile of Group 4 (MRTX-30) against Group 6 (MRTX-30 + Uli-40) showed EGR1 and DUSP6 are among the top differentially expressed genes at day 21 (A, C).
- In the H2122 model, differential expression analysis comparing Group 9 (MRTX-100 + uli-75) vs. Group 5 (MRTX-100), again revealed EGR1 and DUSP6 as a top differential genes (B, D.
- EGR1 and DUSP6 are known ERK1/2 transcriptional targets. The combination of MRTX and uli are synergistic in inhibition of these targets in both models, suggesting superior pathway inhibition with combination treatment compared to single agent MRTX.



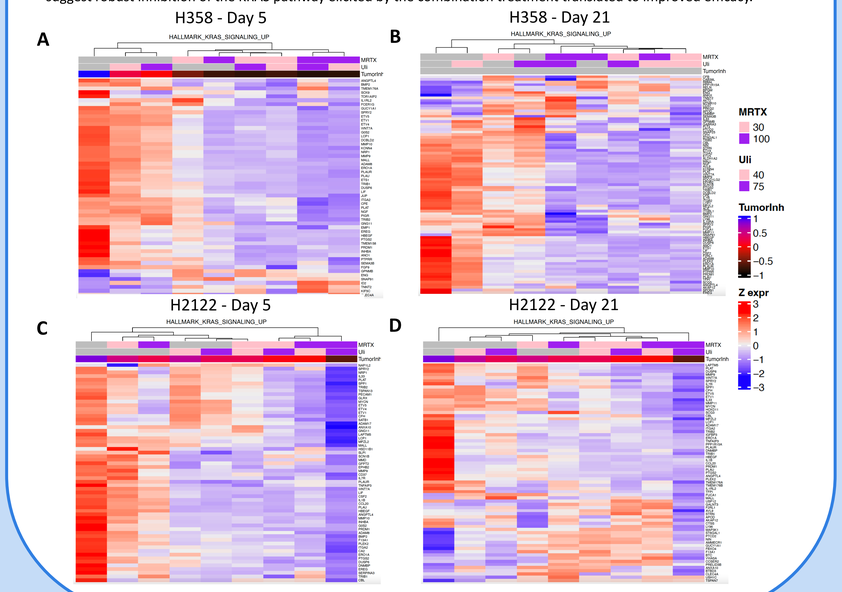
4. A MAPK signature score is correlated with efficacy and is down-regulated in a dose dependent manner



- Durable inhibition of ERK-regulated outputs has been suggested in other studies to be important for maximal response to MRTX849 treatment[7].
- To measure ERK engagement, a MAPK Pathway Activity Score (MPAS)[8] was computed in H358 and H2122 samples collected on day 5 and day 21 of treatment (A). Similar patterns of MPAS reduction were observed at day 5 and day 21.
- In both models, a dose-dependent decrease in MPAS was observed in uli and MRTX treatment groups. In H2122, the benefit of adding ulixertinib to low dose MRTX849 monotherapy observed in panel 1 was also reflected in the MPAS score reduction.
- Combination treatment durably inhibited ERK activity, as indicated by decreased MPAS. MPAS score reduction is correlated with efficacy in both models at day 21 (B).

5. KRAS signaling is synergistically impacted by MRTX849 + uli combination treatment

- To further understand the impact of combining MRTX849 with uli, differential expression analysis was performed on tumors collected on day 5 and day 21 to identify pathways associated with tumor growth inhibition. Consistent with previous studies by others[7], significantly enriched genesets related to KRAS signaling, apoptosis, mTOR, MYC, and cell cycle were revealed. Combination treatment further enriched each of these pathways compared to single agent MRTX849.
- The KRAS signaling up geneset was robustly down-regulated by the MRTX + uli combination groups compared to vehicle in H358 at day 5 (A) and day 21 (B), and in H2122 at day 5 (C) and day 21 (D).
- Reduction of KRAS signaling was clearly dose proportional for both single agent and combination treatment in H2122. A similar trend was observed in the more sensitive model H358. In H358, high- and low-dose combination therapy resulted in KRAS signaling inhibition.
- KRAS signaling inhibition with the MRTX + uli combination is durable, as indicated by comparing day 5 and day 21. These data suggest robust inhibition of the KRAS pathway elicited by the combination treatment translated to improved efficacy.



Conclusions

- Combination treatment of ulixertinib plus MRTX849 resulted in superior tumor growth inhibition compared to dosing of either single agent alone in *KRAS*^{G12C} mutant CDX models. Combination benefit was observed in models with high responsiveness and partial sensitivity to single agent MRTX849.
- Combination treatment resulted in a reduction of MAPK Pathway Activity Score that correlated with efficacy.
- Combining low-dose MRTX849 with either low- or high-dose ulixertinib provided equal to or superior efficacy, respectively, compared to high-dose MRTX849 monotherapy, perhaps providing an avenue to decrease MRTX849 dose without sacrificing efficacy.
- Differential gene expression analysis of samples collected on day 5 and day 21 revealed KRAS gene signaling to be highly significant. High-dose combination treatment robustly inhibited KRAS signaling in H2122. In H358, the combination of high- and low-dose uli + MRTX849 yielded KRAS signaling inhibition and efficacy.
- In summary, ulixertinib combined with MRTX849 (adagrasib) exhibited robust pre-clinical activity in xenograft models with *KRAS*^{G12C} that warrants further evaluation of the combination of ulixertinib with this class of inhibitors.

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Acknowledgments

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