# Combining ulixertinib (ERK1/2 inhibitor) with EGFR and BRAF inhibition yields significant efficacy in preclinical *BRAF*$^{V600E}$ mutant colorectal cancer models

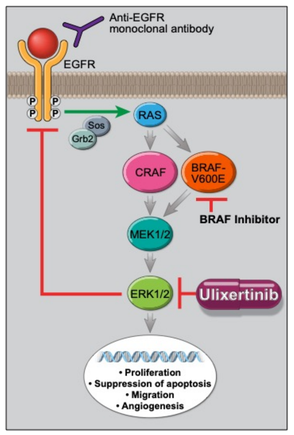
Deborah Knoerzer[1], Anupama Reddy[2], Jessica A. Box[1], Anna Groover[1], Brent Kreider[1], Martin Teresk[1], Caroline M. Emery[1]

[1]BioMed Valley Discoveries, Kansas City, MO [2]Vindhya Data Science Inc., Morrisville, NC

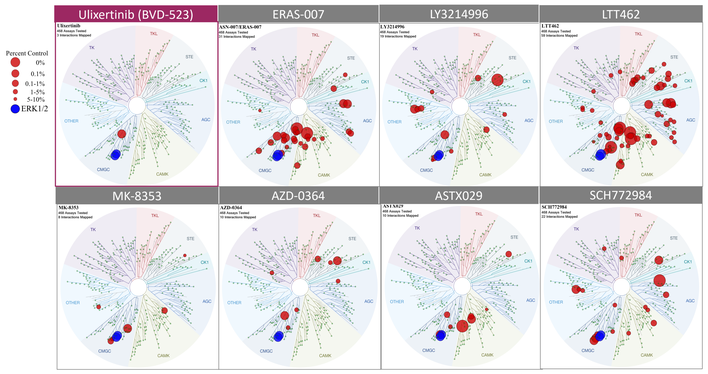
Abstract #2693

## Background

- The *BRAF*$^{V600E}$ mutation occurs in approximately 7% of colorectal cancer (CRC)[1].
- BRAF plus EGFR inhibition (encorafenib with cetuximab) is an FDA approved treatment option for adult patients with metastatic CRC.
- Ultimately, many patients develop resistance leading to disease progression. Adding the MEK inhibitor, binimetinib, has not conferred an overall survival benefit for patients[2].
- Ulixertinib (BVD-523) is a first-in-class and best-in-class small molecule inhibitor of ERK1/2 currently being investigated in several oncology clinical trials (mono- and combination- therapy).
- ERK1/2 are the terminal master regulator kinases of the RAF-MAPK pathway. We hypothesized inhibition of ERK1/2 with BRAF + EGFR inhibition would increase magnitude and duration of response by overcoming MAPK-related acquired resistance mechanisms.



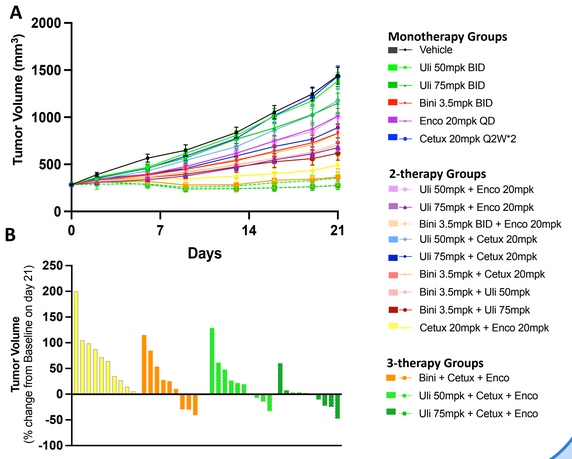

## 1. Ulixertinib - highly selective ERK1/2 inhibitor



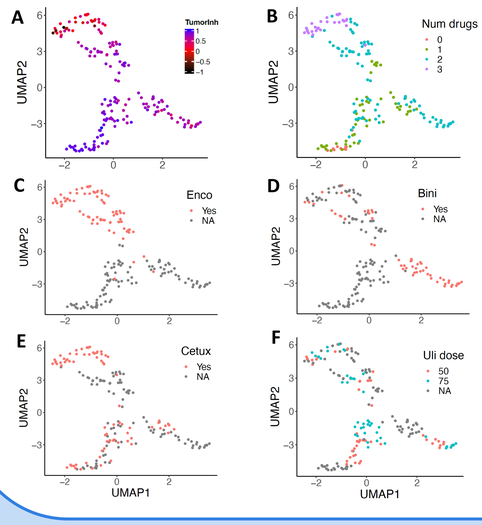

- Ulixertinib inhibits ERK1/2, the terminal master regulator kinase in the MAPK pathway.
- Ulixertinib, in addition to 7 other ERK1/2 inhibitors, were assayed against the KINOMEscan®, an active site directed competition binding assay against 468 kinases.
- Ulixertinib demonstrated a superior selectivity profile compared to all other ERK1/2 inhibitors.

## 2. Ulixertinib in combination with cetuximab and encorafenib is efficacious *in vivo*

- *In vivo* efficacy of combinations of ulixertinib (uli), binimetinib (bini), encorafenib (enco), and cetuximab (cetux), were assessed in the *BRAF*$^{V600E}$ mutant colorectal CDX model HT29 in BALB/c nude mice (A).
- The approved regimen of enco + cetux yielded 71% tumor volume (TV) change from baseline (BL) ($p$=0.001).
- Enco + cetux with uli (50mpk) or bini demonstrated TV % change from BL of 25% ($p$=0.001).
- Tumor regression was observed in high dose uli (75 mpk) + enco + cetux, with an average -3% TV change from BL ($p$=<0.001) (B).

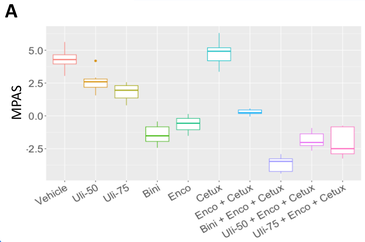



## 3. Gene expression shows clustering by treatment and response




- RNA-sequencing was performed on tumors collected 2-hours post-dose on day 21 and day 42.
- Uniform Manifold Approximation and Projection (UMAP) was applied to the gene expression data to visualize patterns of clustering.
- Tumors clustered by percent tumor growth inhibition (A), and the number of agents combined in the treatment (B). Treatment with 3 agents overlayed with the greatest tumor growth inhibition.
- The majority of tumors receiving enco treatment formed a cluster (C) that overlayed with the greatest percent tumor growth inhibition (A).
- Some clustering was observed with bini treatment (D), but unlike enco, the bini cluster did not overlay with the greatest percent tumor growth inhibition. Cetuximab (E) or ulixertinib (F) treatment did not drive distinct clusters.
- Overall, these patterns establish a framework to elucidate meaningful changes in gene expression with treatment response.

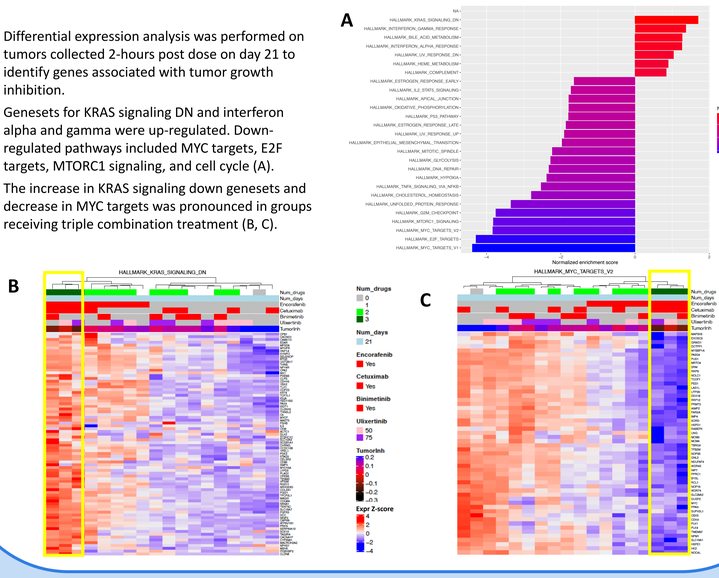
## 4. A MAPK signature score is impacted greatest by triple combination therapy



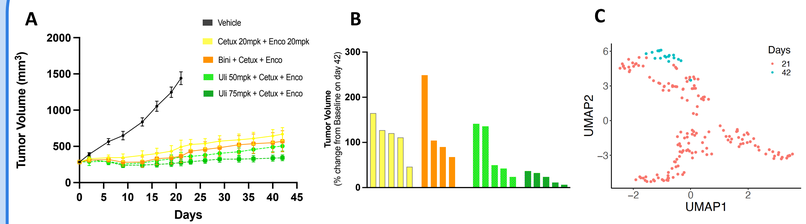

- Changes in the transcriptional MAPK Pathway Activity Score (MPAS)[3] were measured in samples collected at day 21 of treatment.
- Single agent uli at either 50 or 75 mpk, had a modest impact on MPAS compared to single agent bini. MPAS may be biased to MEKi responses since it was derived from MEKi response datasets[3].
- MPAS was most diminished compared to vehicle in groups receiving triple combination treatment. Importantly, triple treatment reduced MPAS to a greater extent compared to the approved regimen of enco + cetux. In triple therapy context, bini impacted MPAS to a greater extend than uli, however response to high-dose uli triple therapy demonstrated superior durability to bini triple therapy (Panel 6).

## 5. Tumor regression is associated with inhibition of MYC and enrichment of KRAS signaling genesets

- Differential expression analysis was performed on tumors collected 2-hours post dose on day 21 to identify genes associated with tumor growth inhibition.
- Genesets for KRAS signaling DN and interferon alpha and gamma were up-regulated. Down-regulated pathways included MYC targets, E2F targets, MTORC1 signaling, and cell cycle (A).
- The increase in KRAS signaling down genesets and decrease in MYC targets was pronounced in groups receiving triple combination treatment (B, C).



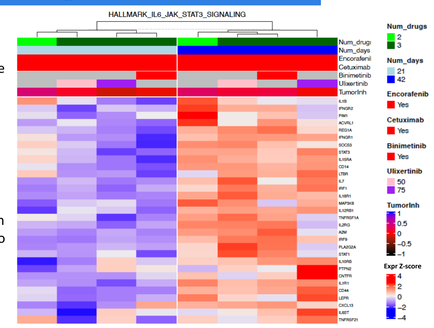

## 6. Efficacy is diminished with time




- It is well documented that acquired resistance to targeted therapeutics diminishes durability of response. Work by others has demonstrated ERK + EGFR + BRAF inhibition in CRC models prevents outgrowth of resistant clones with a cytotoxic effect[4].
- To understand longevity of efficacy, four groups (n=5 per group) continued to dose beyond day 21 until day 42 (A, B). This included the approved regimen of cetux + enco and the groups yielding tumor growth inhibition by day 21.
- With continuous dosing, tumors from all treatment groups increased in volume. Uli (75 mpk + cetux + enco) had the greatest tumor inhibition at day 42 compared to all other treatment groups.
- UMAP of the gene expression data shows samples at day 42 are distinct, suggestive of similar adaptive responses across all treatment groups (C), regardless of the specific combination deployed.

## 7. IL6/JAK/STAT3 enrichment associated with tumor outgrowth

- Gene expression was compared at day 42 vs. day 21 to elucidate potential drivers of tumor growth.
- IL6/JAK/STAT3 pathway was significantly enriched in the 4 treatment groups at day 42 compared to day 21.
- The IL6/JAK/STAT3 geneset started to emerge at day 21 in the doublet combination treatment group of cetux + enco. The pathway is further enriched at day 42 in this treatment group. By day 42, the triple combination of high dose uli with cetux + enco is the most distinct treatment group compared to enco + cetux.
- Over time the IL6/JAK/STAT3 pathway may play a role in adaptive responses to MAPK pathway + EGFR leading to diminished efficacy. STAT3 is known to be activated in CRC, associated with cancer cell proliferation, tumor growth, invasion, migration and resistance to chemoradiotherapy[5].




## Conclusions

- The triple combination of cetux + enco + uli or bini resulted in tumor regression in the *BRAF*$^{V600E}$ mutant CRC model HT29. The triple combination resulted in superior tumor growth inhibition compared to dosing of any single agent or doublet.
- Differential expression analysis demonstrates inhibition of ERK signaling (increase in KRAS signaling DN geneset) is evident with tumor response to therapy, with triple combination therapy resulting in the decreased MPAS.
- In responsive groups, tumors gradually begin to grow out with continuous dosing, suggesting adaptive response. Reactivation of the MAPK pathway has been demonstrated to drive acquired resistance to BRAFi based therapy in CRC[4].
- Gene expression analysis revealed enrichment of IL6/JAK/STAT3 signaling, which may play a role in supporting tumor growth in the face of EGFR/MAPK pathway inhibition.
- Based in part on the data shown here, along with the previous clinical observation that compassionate use in a patient with metastatic CRC treated with uli + cetux + enco resulted in a complete response[6], a clinical trial of uli + cetux ± enco for the treatment of patients with unresectable metastatic CRC is planned.

## References

1. *BRAF*$^{V600E}$ prevalence in CRC patient samples from cBioportal:
   - Cerami et al. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discovery. May 2012 2; 401.
   - Gao et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 6, pl1 (2013).
2. Tabernero et al. (2021). Encorafenib Plus Cetuximab as a New Standard of Care for Previously Treated BRAF V600E–Mutant Metastatic Colorectal Cancer: Updated Survival Results and Subgroup Analyses from the BEACON Study. *Journal of Clinical Oncology*, *39*(4), 273-284.
3. Wagle MC et al. A transcriptional MAPK Pathway Activity Score (MPAS) is a clinically relevant biomarker in multiple cancer types. NPJ Precis Oncol. 2018 Mar 7;2(1):7.
4. Hazar-Rethinam et al. Convergent Therapeutic Strategies to Overcome the Heterogeneity of Acquired Resistance in *BRAF*$^{V600E}$ Colorectal Cancer. Cancer Discov. 2018 Apr;8(4):417-427.
5. Gargalionis AN, Papavassiliou KA, Papavassiliou AG. Targeting STAT3 Signaling Pathway in Colorectal Cancer. Biomedicines. 2021 Aug 15;9(8):1016.
6. Stuhlmiller et al. Updated clinical outcomes from ULI-EAP-100, an intermediate expanded access program for ulixertinib (BVD-523). 2022 ASCO Meeting Abstract.

## Acknowledgments

- Crown Biosciences for CDX experiments.
- Azenta Life Sciences for RNA-sequencing.
- Vinodh Srinivasan and Alex Nesta from Vindhya Data Science for RNA-seq data processing.
- Mark Miller (Stowers Institute for Medical Research, Kansas City, MO) for pathway illustration.