The Characterization of In Vitro Models Demonstrating a Significant Delay of Acquired Resistance to Ulixertinib (ERK1/2)

Deborah Knoerzer[1], Anupama Reddy[2], David Sorrell[3], Caroline M. Emery[1]

[1]BioMed Valley Discoveries, Inc., Kansas City, MO. [2]Vindhya Data Science Inc., Morrisville, NC. [3]Horizon Discovery Ltd., Cambridge, United Kingdom

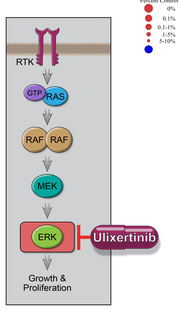
BioMed Valley Discoveries

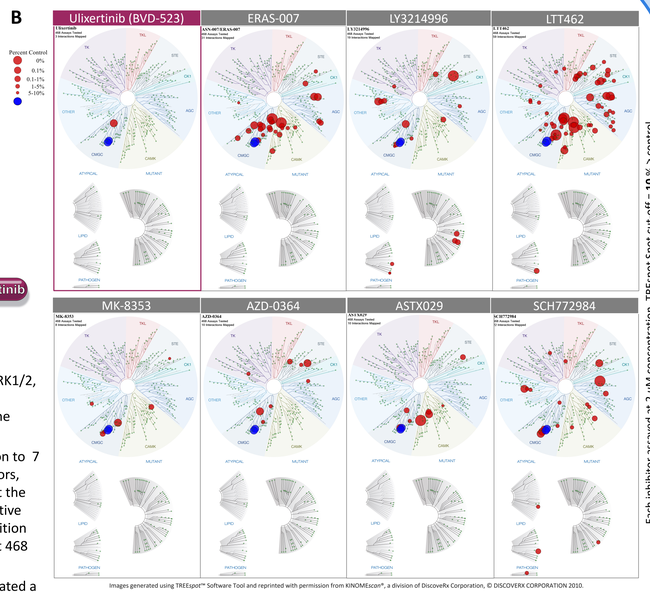
Abstract #415

Background

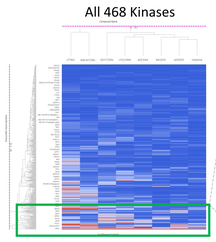
- Ulixertinib (BVD-523) is a first-in-class small molecule inhibitor of ERK1/2 currently being investigated in several oncology clinical trials, both as a single agent, and in combination with other anti-cancer therapeutics[1,2].
- Clinical acquired resistance has been described by others for numerous efficacious small molecule agents. Relevant to the MAPK pathway, emergence of resistance to BRAF and MEK inhibitors limits their clinical efficacy.
- In vitro models of ulixertinib resistance were generated in an endeavor to characterize and predict potential mechanisms of resistance and guide rational combination therapies. The BRAF-V600E mutant melanoma cell line, A375, was cultured in progressively increasing concentrations of ulixertinib or other MAPK pathway inhibitors (dabrafenib and/or trametinib).
- To elucidate potential mechanisms of resistance, Reverse Phase Protein Array (RPPA) profiling of 306 proteins was performed on parental A375 and ulixertinib resistant clones following treatment with ulixertinib at varying concentrations and treatment times.

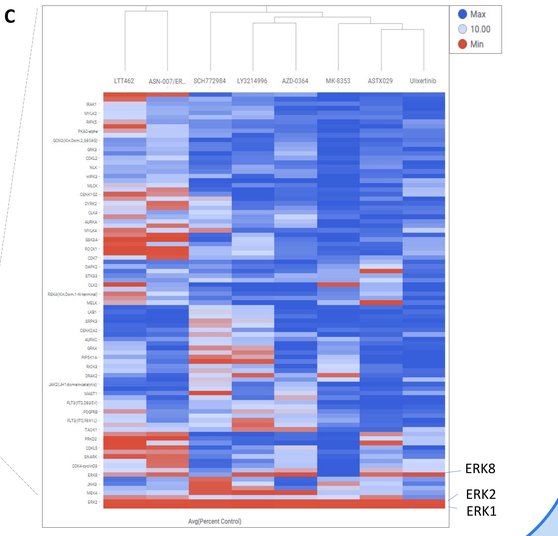
1. Ulixertinib is a selective ERK1/2 inhibitor





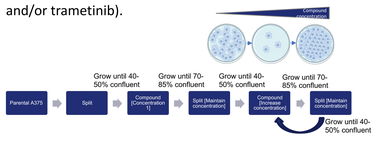
- Ulixertinib inhibits ERK1/2, the terminal master regulator kinase in the MAPK pathway (**A**).
- Ulixertinib, in addition to 7 other ERK1/2 inhibitors, were assayed against the KINOMEscan®, an active site directed competition binding assay against 468 kinases (**B**).
- Ulixertinib demonstrated a superior selectively profile compared to the other ERK1/2 inhibitors (**B, C**).
- In addition to binding to ERK1/2, ulixertinib binds to ERK8 in this assay; 0.15, 0.3 and 0.45 % control, respectively.
- ERK 8 is a common 'off-target' of ERK 1/2 inhibitors (**C**).

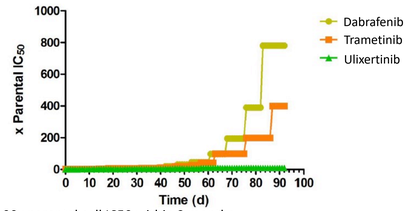




2. Generating MAPK pathway inhibitor resistant clones

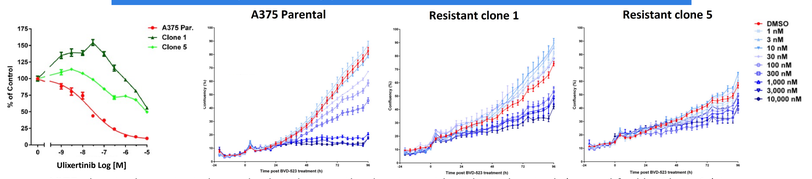
- The BRAF-V600E mutant melanoma cell line, A375, was cultured in progressively increasing concentrations of ulixertinib or other MAPK pathway inhibitors (dabrafenib and/or trametinib).





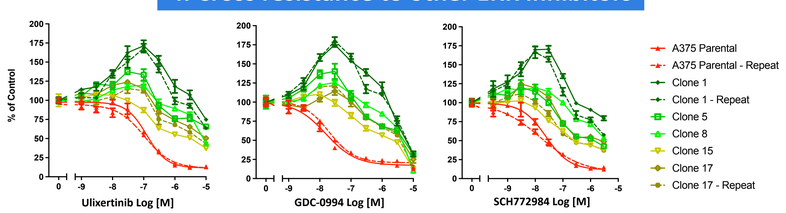
- Dabrafenib (BRAFi) or Trametinib (MEKi) culturing achieved 100x parental cell IC50 within 2 months.
- > 3 months of BVD-523 culturing to achieve growth in 15x parental IC50. 10 ulixertinib resistant clones were expanded for characterization.

3. Growth phenotype of ulixertinib resistant clones



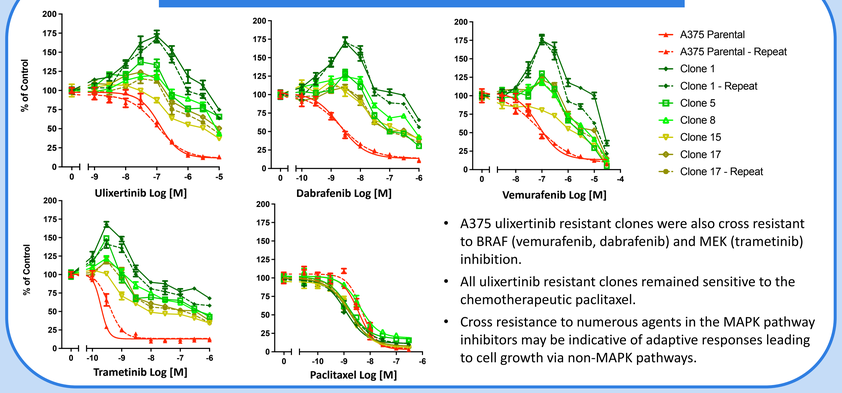
- A375 ulixertinib resistant clones displayed a growth advantage at low dose ulixertinib (exemplified by Clone 1 (strong phenotype), and Clone 5 (weak phenotype)).
- Increasing ulixertinib concentration overcame the growth induction phenotype.

4. Cross resistance to other ERK inhibitors



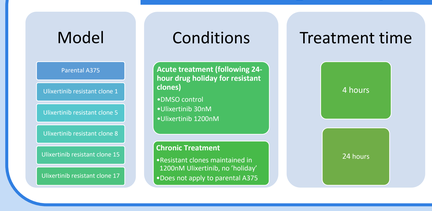
- A375 ulixertinib resistant clones were cross-resistant to other ERK inhibitors, including all compounds indicated in panel #1 (growth inhibition curves for GDC-0994 and SCH772984 shown here).
- Low dose treatment with all MAPK pathway inhibitors tested in these experiments conveyed a growth advantage.

5.Cross resistance to MEK and BRAF inhibitors

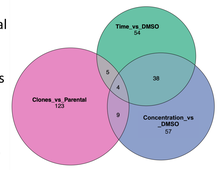


- A375 ulixertinib resistant clones were also cross resistant to BRAF (vemurafenib, dabrafenib) and MEK (trametinib) inhibition.
- All ulixertinib resistant clones remained sensitive to the chemotherapeutic paclitaxel.
- Cross resistance to numerous agents in the MAPK pathway inhibitors may be indicative of adaptive responses leading to cell growth via non-MAPK pathways.

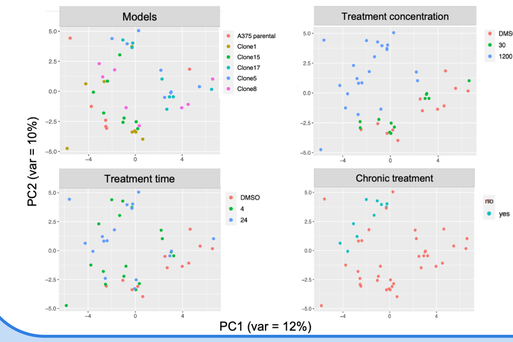
6. Generating a sample matrix for RPPA analysis



- Samples for RPPA were generated from parental A375, and 5 ulixertinib resistance clones, following the conditions and treatment times outlined.
- Samples were assayed against 305 proteins by RPPA.



7. PCA indicates clusters by treatment condition



- Principal Component Analysis (PCA) was performed using the RPPA features and plotted to show different covariates (colors) in the sub-figures.
- Treatment with high concentrations of ulixertinib clustered with chronic ulixertinib treatment.
- At low ulixertinib concentrations, 2 clusters were revealed:
  - Parental, clone1, clone15
  - Clone17, clone5

8. RPPA analysis reveals dynamic signaling changes in ulixertinib resistant clones

- Differential gene expression analysis revealed enrichment of several pathways in ulixertinib resistant clones (Reactome gene families shown).
- Of note, enrichment of PI3K and AKT pathway genes was observed **(A)**.

Numerous protein changes are enriched in ulixertinib resistant clones **(B)**. Interesting trends include:

- Components of MAPK signaling remain inhibited in the ulixertinib resistant setting.
- A rewiring to HER2 signaling from EGFR observed in ulixertinib-resistant clones (chronic or high concentration ulixertinib treatment). This is consistent with work presented by others (Abstract #5333).
- Markers of autophagy enriched in ulixertinib resistant clones. A Phase II clinical trial to assess the combination of ulixertinib and autophagy inhibition (Hydroxychloroquine) is ongoing (NCT05221320).
- FAK and STAT3 represent interesting potential targets in the ulixertinib resistant setting.

Conclusions

- Ulixertinib is a selective ERK1/2 inhibitor, with a superior kinase selectivity profile compared to other ERK inhibitors.
- Drug resistant A375 clones were readily obtained following growth in high concentrations of dabrafenib or trametinib. In contrast, developing resistance to ulixertinib proved challenging. This may translate to improved durability of response to ulixertinib in the clinic.
- A375 ulixertinib resistant clones displayed a growth advantage at low concentrations of MAPK pathway inhibitors (BRAFi, MEKi, and ERKi). The mechanism of this phenomenon remains to be elucidated. Increasing compound concentration reverses this growth phenotype.
- Differences between parental A375 and ulixertinib resistant clones were revealed, including components of MAPK, HER2, FAK, STAT3, and autophagy markers. This work begins to tease out potential mechanisms of resistance to ulixertinib and guide potential combination partners that could circumvent acquired resistance. Further interrogation of ulixertinib resistant clones by transcriptomics has identified potential combinations partners to combat acquired resistance (see Abstract #404).

References

1. Germann et al, 2017. Targeting the MAPK Signaling Pathway in Cancer: Promising Preclinical Activity with the Novel Selective ERK1/2 Inhibitor BVD-523 (Ulixertinib). Mol Cancer Ther. 2017 Nov;16(11):2351-2363.
2. Sullivan et al, 2018. First-in-class ERK1/2 inhibitor ulixertinib (BVD-523) in patients with MAPK mutant advanced solid tumors: Results of a phase I dose-escalation and expansion study. Cancer Discov. 2018;8(2):184-195.

Acknowledgments

- BioMed Valley Discoveries team members (Anna Groover, Martin Teresk, Brent Kreider, Jessica Box, Lisa Lassise).
- Mark Miller (Stowers Institute for Medical Research, Kansas City, MO) for pathway illustrations.
- Reverse Phase Protein Array (RPPA) Core Facility at MD Anderson Cancer Center.
- Horizon Discovery Cell Panel Screening Group.