Identification of Combination Partners to Combat Acquired Resistance to Ulixertinib (ERK1/2 inhibitor) Using Transcriptomics

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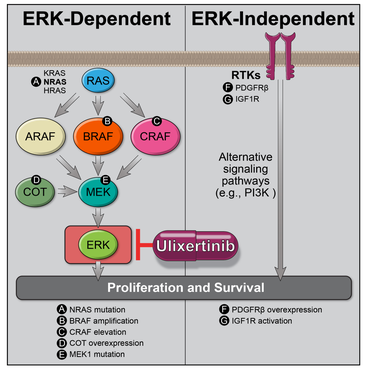
BioMed Valley Discoveries

Abstract #404

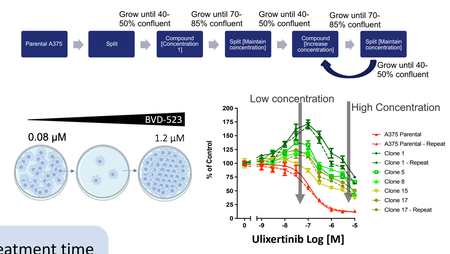
Background

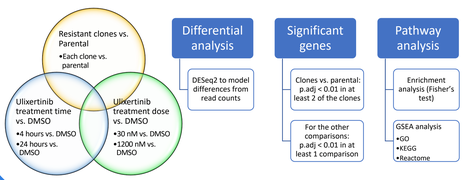
- Ulixertinib (BVD-523) is a first-in-class and highly selective small molecule inhibitor of ERK1/2 currently being investigated in several oncology clinical trials, both as a single agent, and in combination with other anti-cancer therapeutics[1,2] .
- Drug resistance is the rate-limiting step in the successful clinical utility of MAPK inhibitors.
- Mechanisms of resistance to BRAF and MEK inhibitors include feedback mechanisms and compensatory pathways (adaptive responses)[1].
- Ulixertinib resistant clones were generated and characterized by RNA sequencing to help predict mechanisms of resistance and guide rational combination therapies.



Methods

- In vitro experiments were deployed to develop models resistant to ulixertinib (**see abstract #415**).
- Ulixertinib resistant clones were generated by culturing the A375 cell line (melanoma; BRAF V600E) in escalating concentrations of ulixertinib.

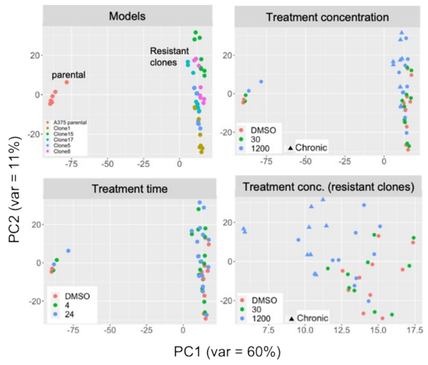




- RNA-sequencing was performed on the A375 parental model and the ulixertinib resistant clones following varying treatment conditions with ulixertinib (total N = 46):
  - Controls (treated with DMSO)
  - Dose (low and high concentration)
  - Time (4 and 24 hours)
  - Chronic treatment
- Differential analysis of RNA-seq data was performed by comparing:
  - Resistant clones to the parental models
  - Ulixertinib treatment times vs. controls
  - Ulixertinib treatment conc. vs. controls

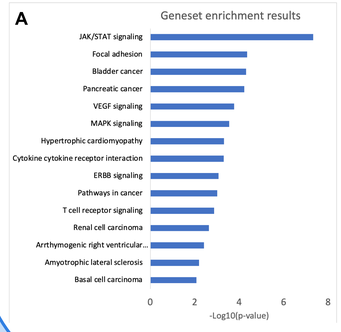
1. Transcriptomic analysis highlights an ulixertinib concentration dependent resistance phenotype

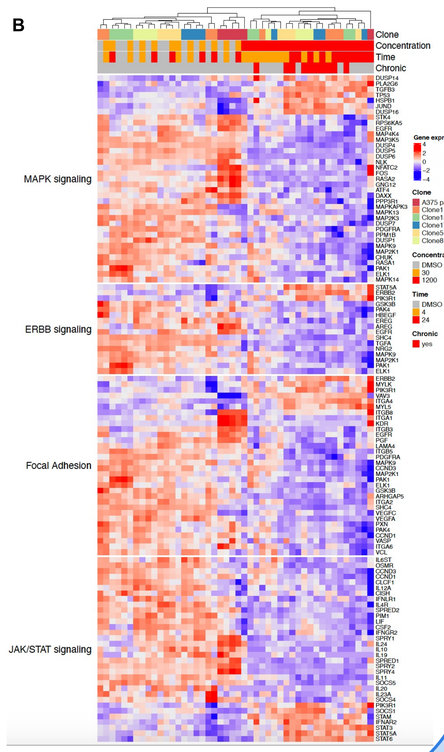
- RNA-sequencing and principal component analysis revealed strong differences between parental and ulixertinib resistant clones.
- There is further differentiation between acute high ulixertinib concentration and chronic ulixertinib treatment models, compared to models treated with acute lower concentrations of ulixertinib.



2. Resistance associated pathways include MAPK, autophagy, focal adhesion, PI3K/AKT

- Differential gene expression analysis revealed enrichment of several pathways, including MAPK, ERBB, focal adhesion, and JAK/STAT **(A, B)**. These data are consistent with experiments published by Ito et al., they described MAPKi resistant cells have markedly increased baseline levels of MAPK signaling[4].
- We observed rewiring of PI3K/AKT by shifting the signaling from EGFR to ERBB2 in the ulixertinib resistance models **(B)**.
- The magnitude of changes in these genes and pathways also exhibited an ulixertinib concentration-dependent effect, with higher concentration treatment models showing a higher fold-change compared to the lower concentration treated models **(B)**.



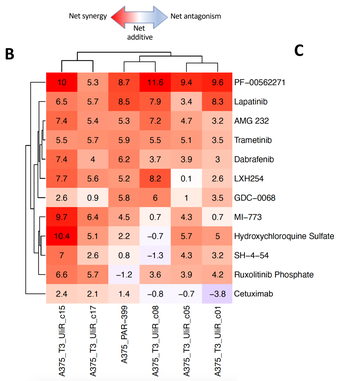


3. Inhibitor combination experiments validate hypotheses from differential gene expression

- To validate hypotheses generated from the differential gene expression analysis of ulixertinib resistant clones, we performed an in vitro inhibitor combination screen in the parental A375 cell line and ulixertinib resistant clones **(A)**. Inhibitor combination testing was performed with background ulixertinib treatment, due to the MAPK inhibitor growth addiction phenotype of ulixertinib resistant clones (**see abstract #3545**).
- Synergy was computed using the Lowe model using growth inhibition dose matrices and is shown as a heatmap **(B)**. Synergistic targets suggested by gene expression analysis were validated in the combination experiments.
- Robust synergy was observed between ulixertinib plus FAK (focal adhesion) inhibition **(B, C).**
- Synergies between ulixertinib and lapatinib (ERBB2 antagonist) in the ulixertinib resistant clones were demonstrated, but not with cetuximab (EGFR antagonist), consistent with expression of each inhibitor's target **(B, C)**.

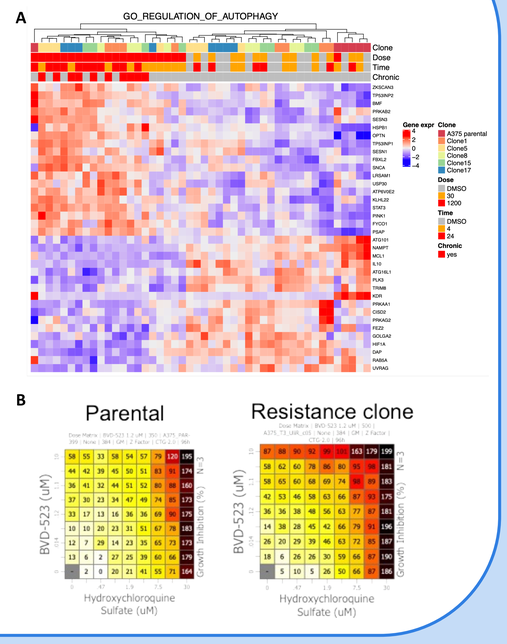
A

Compound	Target
Ulixertinib	ERK1/2
Ruxolitinib	JAK
AMG-232	MDM2
MI-773	MDM2
Lapatinib	ERBB2 and EGFR
Cetuximab	EGFR
Hydroxychloroquine (HCQ)	Autophagy
Trametinib	MEK1/2
Dabrafenib	BRAF
GDC-0068	AKT
PF-562271	FAK
LXH254	pan-RAF
SH-4-54	STAT3



4. Autophagy inhibitor (HCQ) is a synergistic drug combination with ulixertinib

- Statistically significant gene expression changes indicative of autophagy were observed **(A)**.
- The interplay between the MAPK pathway and autophagy has been described by others in pancreatic models[5]. The combination of ulixertinib plus hydroxychloroquine (HCQ) in KRAS mutated PDAC cells demonstrated ERK inhibition increased cellular dependence on autophagy for survival[5].
- These experiments validated the combination of ulixertinib with autophagy inhibitor HCQ. Synergies were observed both in parental A375 and ulixertinib resistant clones **(B)**.
- The combination of ulixertinib plus hydroxychloroquine is currently under clinical investigation: Phase I (NCT04145297) and Phase II clinical trials (NCT05221320).



Conclusions

- Ulixertinib is a selective ERK1/2 inhibitor. Drug-resistant A375 clones were readily obtained following growth in high concentrations of MAPK pathway inhibitors dabrafenib (BRAFi) or trametinib (MEKi). In contrast, developing resistance to ulixertinib proved challenging (**see Abstract #415**).
- When ulixertinib resistant clones were finally generated, RNA sequencing analysis of the resistant clones compared to parental A375 revealed similar changes in genes and pathways across the different resistant clones.
- Differential pathways included MAPK, ERBB, focal adhesion, JAK/STAT, and VEGF. A rewiring from EGFR signaling to ERBB2 was observed in the ulixertinib acquired resistant clones.
- The hypotheses generated from gene expression analysis were validated by inhibitor combination screens. Strong synergy was observed with FAK (focal adhesion) inhibition and ERBB2 inhibition (lapatinib).
- The combination of ulixertinib and EGFR antagonist (cetuximab) was not synergistic in the ulixertinib resistant setting. This was consistent with the observation of low EGFR expression.
- The autophagy inhibitor, hydroxychloroquine (HCQ), exhibited synergy with ulixertinib and is currently being evaluated in clinical trials.

References

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Acknowledgments

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