Significant Efficacy Demonstrated With the Combination of Ulixertinib (ERK1/2 Inhibitor) and CDK4/6 Inhibitors in MAPK Altered Models

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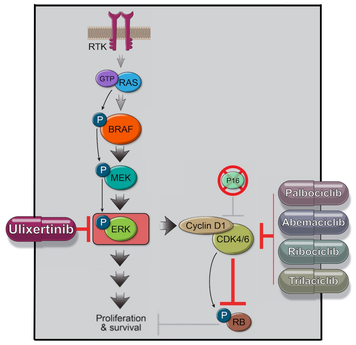
Background

- Ulixertinib (BVD-523) is a first-in-class small molecule inhibitor of ERK 1/2 currently being investigated in several oncology clinical trials, both as a single agent and in combination with other anti-cancer therapeutics[1,2].
- Palbociclib and ribociclib are FDA approved orally active, potent, and highly selective reversible inhibitors of the CDK4 and CDK6 kinases.
- It has been well established that ERK activation increases cyclin D1 levels and entry into the cell cycle[3]. The combination of MEKi with CDK4/6i has been shown to be beneficial[4] and has since been tested in the clinic.
- We hypothesized that the combination of ERK1/2 and CDK4/6 inhibitors would have synergistic antitumor activity and cause tumor regression in vivo.



Methods

In Vitro

- Combination interactions across a dose matrix of concentrations were determined by the Loewe Additivity and Bliss Independence models using Horizon's Chalice™ Bioinformatics Software. Synergy was visualized by displaying the calculated excess inhibition over that predicted as being additive at each test point across the matrix as a heat map.
- Activity over Loewe additivity can be quantified in Chalice™ using a simple volume score, which effectively calculates a volume between the measured and Loewe additive response surfaces and emphasizes the overall synergistic (positive values) or antagonistic (negative values) effect of the combination.

In Vivo xenograft models

- Female athymic nude mice (Crl:NU(Ncr)-Foxn1nu, Charles River) were implanted subcutaneously with cells (SW620/HCT116) or tumor fragments (MiaPaCa-2/ME-010). Animals were fed *ad libitum* water and a modified and irradiated lab diet; animals were housed on irradiated bedding in static microisolators and maintained on a 12-hr light cycle. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC).
- Two hours after the final dose, samples were taken from all animals in all groups. Tumors were excised and snap frozen. Samples were stored at -80 °C until shipment to specified vendors for analysis.

Reverse Phase Protein Array (RPPA)

- Tumors were processed as formalin fixed paraffin embedded (FFPE) or flash frozen (FF) specimens and laser capture microdissection (LCM) was conducted and prepared for RPPA for a total of 24 selected proteins.

1. ERK and CDK4/6 inhibitors single agent IC50s across a panel of four lung cancer cell lines

- The two cell lines carrying a KRAS mutation are more sensitive to ulixertinib relative to the KRAS wildtype cell lines.
- The pattern of response to ERK inhibitor SCH772984 was broadly similar to ulixertinib.

Compound	Relative IC50 (μM)							
	A549 (KRAS G12S)		H2122 (KRAS G12C)		H1437 (KRAS wt)		H226 (KRAS wt)	
	CellTiter	Hoechst	CellTiter	Hoechst	CellTiter	Hoechst	CellTiter	Hoechst
Ulixertinib	0.73	0.59	0.45	0.45	1.2	1.4	34%@10μM	58%@10μM
SCH772984	1.1	0.74	0.63	0.53	57%@3μM	63%@3μM	35%@3μM	49%@3μM
Palbociclib	41%@3μM	0.13	48%@3μM	0.15	29%@3μM	0.22	20%@3μM	0.056
Ribociclib	49%@10μM	0.7	44%@10μM	0.45	30%@10μM	2.8	32%@10μM	0.37

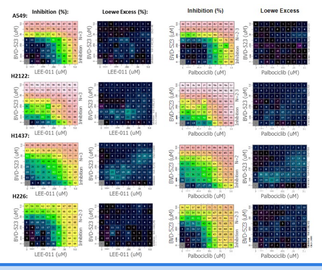
Note: Maximal percentage inhibition are reported when a cell line is relatively insensitive to compound resulting in a partial response (defined as ≤ 60% inhibition achieved) and/or the bottom of the curve not being defined.

- The single agent results for the CDK4/6 inhibitors were dependent on the readout for cell viability, appearing markedly more sensitive to inhibition when assessed using Hoechst staining. This suggests that measurement of ATP levels is not a suitable proxy for the number of viable cells in response to CDK4/6 inhibitors, therefore only the Hoecht stain readout was used for the combination assays.

2. Combination of ulixertinib and CDK 4/6 inhibitors demonstrate additive to modest synergy in vivo

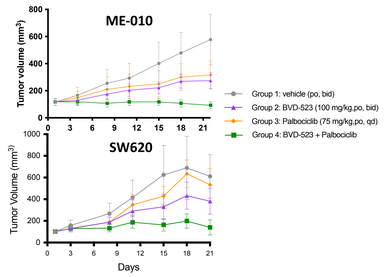
- The combination of ulixertinib plus CDK4/6 inhibitors was examined in lung cancer cell lines.
- Visualization of the Loewe 'excess inhibition' heat maps suggest that the combination of ulixertinib (BVD-523) with either of the two CDK4/6 inhibitors was at least additive, and in some cases potentially synergistic.
- Similar results were obtained with the ERK inhibitor SCH772984 (data not shown).

*Warmer/brighter colors = greater activity



3. Combination of ulixertinib and palbociclib demonstrate significant efficacy in vivo

- **Four xenograft models were utilized to assess the combination of ulixertinib and palbociclib, each xenograft harboring a RAS mutation.**

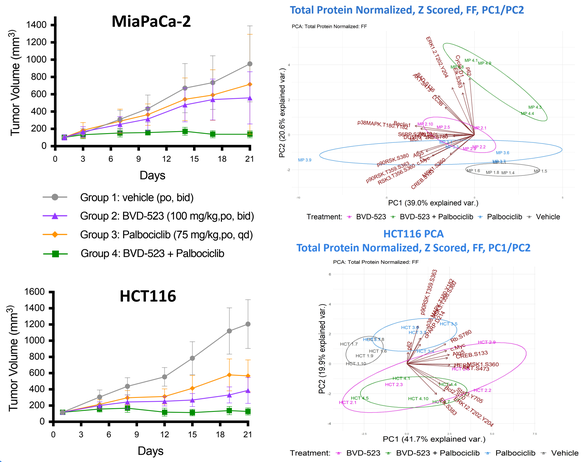


Xenograft Models	MAPK Pathway Mutation	% Tumor Growth Inhibition		
		Ulixertinib	Palbociclib	Combination
		100mpk, PO BID	75mpk, PO QD	100mpk/75 mpk BID, QD
ME-010 (Melanoma)	NRAS p.Q61R	51***	39**	85***
HCT116 (Colorectal)	KRAS p.G13D	71***	51***	90***
SW620 (Colorectal)	KRAS p.G12V	30*	18^{NS}	75***
MiaPaCa-2 (PDAC)	KRAS p.G12C	41*	43^{NS}	83^{NE}

Note: NE = non evaluable, NS = not significant.
* = P<0.05, ** = P < 0.01, *** = P < 0.001 compared to Vehicle Group.

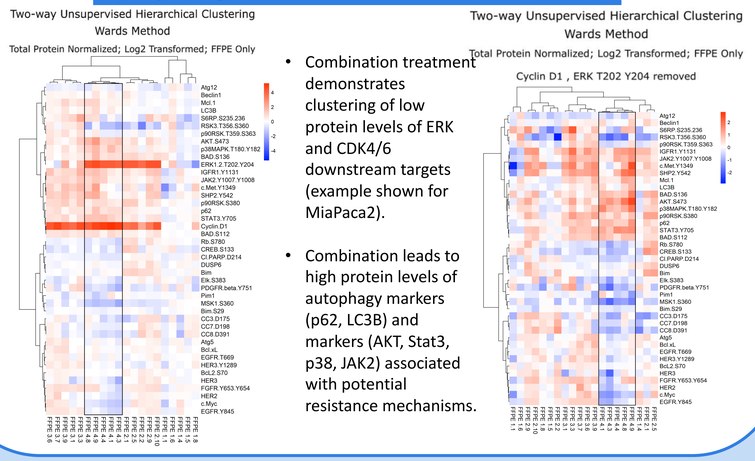
- Ulixertinib monotherapy across all models showed modest tumor growth inhibition (TGI) while palbociclib monotherapy showed limited TGI across all models.
- The combination groups demonstrated significant responses ranging from 75% - 90% TGIs. All treatment regimens were well tolerated across all models.

4. RPPA - principal component analysis (PCA) highlights combination groups as distinct



- PCA indicates the combination treated group in all models tested demonstrates a defined cluster.
- Dominate features of this component for the combination treated groups are ERK T202, p38 MAPK T180 Y182, AKT S473, ELK S383, Cyclin D, p62, LC3B, and Stat3 Y705.

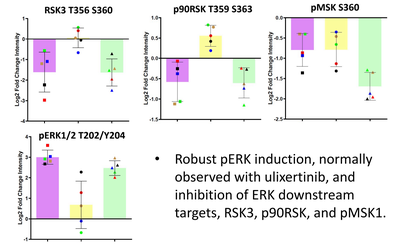
5. RPPA - heat map depiction reinforces unique signature of combination treatment



- Combination treatment demonstrates clustering of low protein levels of ERK and CDK4/6 downstream targets (example shown for MiaPaca2).
- Combination leads to high protein levels of autophagy markers (p62, LC3B) and markers (AKT, Stat3, p38, JAK2) associated with potential resistance mechanisms.

6. RPPA – representative markers of target inhibition and adaptive response

Target inhibition by ulixertinib

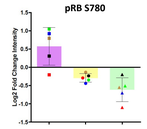


- Robust pERK induction, normally observed with ulixertinib, and inhibition of ERK downstream targets, RSK3, p90RSK, and pMSK1.

Treatment Groups

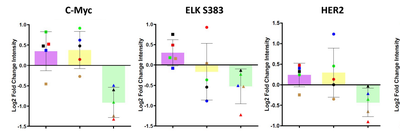
Ulixertinib (100 mg/kg BID) | Palbociclib (75 mg/kg QD) | Ulixertinib + Palbociclib

Target inhibition by palbociclib



- Reduction of pRB S780 was observed with palbociclib.
- Further exacerbation observed in combination with ulixertinib.

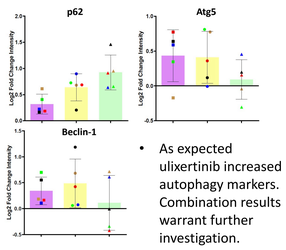
Ulixertinib plus palbociclib combination benefit



- Compared to either monotherapy, the combination of ulixertinib and palbociclib confers superior reduction of key targets (e.g., c-Myc, Elk S383).
- Increased HER2 activity is a known mechanisms of MAPKi resistance, the combination confers reduction of HER2, in addition to HER3.

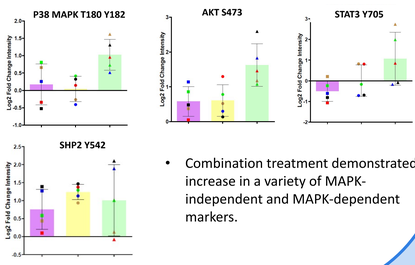
Adaptive responses observed with combination therapy

Autophagy



- As expected ulixertinib increased autophagy markers. Combination results warrant further investigation.

Potential resistance pathways



- Combination treatment demonstrated increase in a variety of MAPK-independent and MAPK-dependent markers.

% change from vehicle, normalized to total protein in either FFPE or FF samples.

Conclusions

- Results from lung cancer cell lines, either wild-type or mutated for KRAS, demonstrated at least additive and in some cases potentially synergistic interactions between ulixertinib and CDK4/6 inhibitors.
- The in vivo efficacy demonstrated by the combination of ulixertinib and palbociclib in a variety of tumor xenograft models was significant.
- Using RPPA, treatment effects on signaling was evaluated in the MAPK family, cell cycle regulation, and other associated feedback and compensatory pathways. Notably, suppression of protein targets downstream of ERK1/2 were observed in both ulixertinib monotherapy and in combination treatment. Similarly, the combination therapy treatment group reduced protein levels involved in cell cycle progression, which was not seen in either monotherapy alone.
- The combination doses used for these studies are known to be in the clinically efficacious range. Due to the clinical relevance of this combination, it will be important to investigate the variety of adaptive response pathways further.
- The efficacy demonstrated with this preclinical work has proven to be translatable to the clinic as the combination of ulixertinib and palbociclib recently achieved MTD in a Phase I trial in advanced solid tumors including pancreatic cancer (NCT03454035). The combination of ulixertinib with an autophagy inhibitor (hydroxychloroquine) is currently being tested in a Phase II trial in patients with advanced gastrointestinal malignancies (NCT05221320).

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