Profiling adaptive responses of renal cell cancer to cabozantinib in order to develop rational drug combinations

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Poster # 11P

BACKGROUND

- Structural adaptive responses (i.e. secondary to intrinsic genetic modifications) to molecularly target treatments are a frequent and well-known escape mechanism in cancer cells.
- Functional adaptive responses (i.e. due to intrinsic cellular machinery modulations) could contribute to target treatment resistance
- Development of targeted treatment combinations for treating RCC is an unmet need.
- We hypothesize that understanding tumor cell adaptive responses induced by cabozantinib (C) in renal cell cancer (RCC) cells could provide a rationale base for selecting treatment combinations and optimise drug doses and schedules.

METHODS

- We evaluated functional proteomic changes induced in VHL-mutated 786-O RCC cell line after *in vitro* exposure to low-dose C using reverse phase protein array (RPPA).
- We exposed 786-O cells to HGF alone or in combination with C at 40 nM for 24 hours and then we performed RPPA analysis.
- A linear model analysis was performed on normalized intensity data from RPPA, in order to identify proteins and phosphoproteins undergoing significant treatment-induced changes.
- Then, we evaluated *in vitro* the efficacy of the interaction between C and drugs selected on the basis of RPPA analysis by mean of dose matrix tests.

RESULTS

- Despite the low dose of C used, we observed a significant variation (i.e. with a log2 fold change for intensity of < 0.7 or > 1.5) in expression or phosphorylation of several protein targets.
- We observed inhibition of protein phosphorylation downstream of C targets (among which AXL, VEGFR−2, components of the PI3K/AKT/mTOR pathway and MEK1), which validates the cell model.
- Unexpectedly, we detected a significantly increased intensity signal for several protein targets involved in DNA repair process (RBBP8, RPA32_pS4_S8, BABAM1, BAP1, CDKN1A).
- We thus tested *in vitro* the association of C and inhibitors of the DNA repair proteins ATM, ATR and Wee1. We could observe that while the combination of C with KU60019 (an ATM inhibitor) is additive, the combinations of C with VE822 (an ATR inhibitor) or MK1775 (a Wee1 inhibitor) are synergistic in 2D and 3D cell culture.

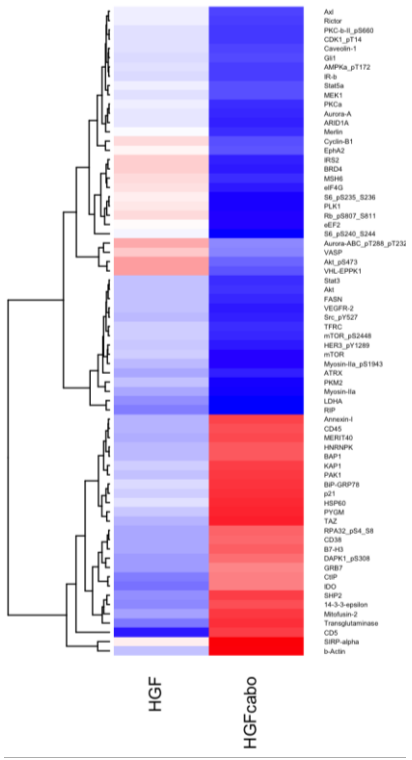

Figure 1 RPPA analysis of protein and phosphoprotein targets. 7866-O cells were treated with HGF or HGF and cabozantinib. The heatmap represents the targets for which the log2 fold change of intensity was < 0.7 or > 1.5.

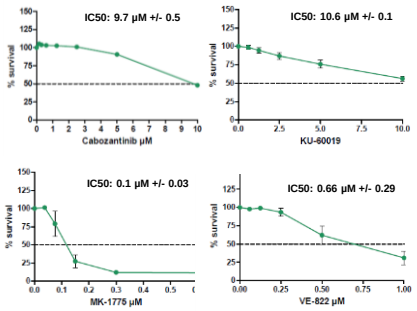

Figure 2 Cytotoxic assay and IC50 of 786-O RCC cells (VHL mutated) treated with cabozantinib, ATM inhibitor KU-60019, WEE1 inhibitor MK-1775 and ATR inhibitor VE-822.

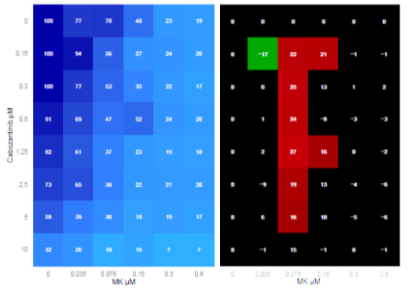

Figure 3 Synergy matrices of cabozantinib + MK-1775 on 786-O RCC cells in 3D culture (spheroids). Blue matrices shows cell viability and black/red/green matrices show interaction effect,bwith Black representing additivity, red synergy and green antagonism.

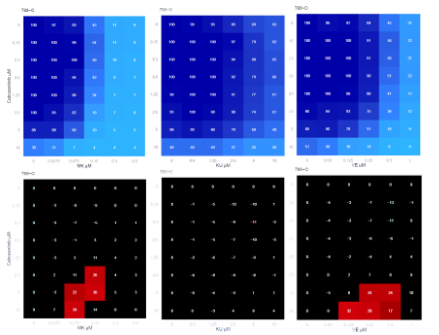

Figure 4 Interaction effect matrices of drug combination on 786-O RCC cells. Blue matrices shows cell viability and black/red/green matrices show interaction effect with black representing additivity, red synergy and green antagonism. Combinations used are from left to right cabozantinib + MK-1775, cabozantinib + KU-60019 and cabozantinib + VE-822.

CONCLUSIONS

- We were able to show that cabozantinib activates DNA damage signaling, and that combination of cabozantinib with inhibitors of proteins involved in DNA damage are synergistic.
- Analysis of functional proteomic changes induced *in vitro* by C helped us to select targets for combination targeted therapy in RCC.
- Overall, our data suggest that cellular adaptive responses to drugs play a role in tumor resistance, and that elucidating them could help in designing drug combinations suitable for testing in the clinical setting.

The study presenter does not declare any significant conflict of interest.
This study was funded by a grant from Ipsen.