

Multimodality Imaging End-Points on mTOR and HSP Inhibition in Pancreatic Cancer: A Pre-Clinical PET/MRI/MRS Study

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Introduction: Pancreatic cancer is the 4th leading cause of cancer death in the United States, with a 5-year survival rate of 4.8%-the lowest of any cancer sub-type. There is an urgent need to develop novel therapeutic strategies utilizing signal transduction inhibitors (STIs) to treat pancreatic cancer. One high-yield target is mTOR, a kinase in the Akt/PI3k signaling pathway that is commonly constitutively-active in pancreatic cancer. mTOR plays a significant role in regulating cell metabolism, proliferation, autophagy, and is strongly linked to cancer cell survival. Clinical evaluations of mTOR inhibitors have not demonstrated any efficacy. This project utilizes a combination treatment of everolimus (RAD001), an mTOR inhibitor, and a second generation HSP-90 inhibitor, ganetespiib, in everolimus resistant pancreatic cancer. HSP-90 affects multiple signaling pathways (i.e. c-Kit, EGFR, and Bcr-Abl) that help the devolvement of everolimus resistance. This study aims to establish metabolic (FDG-PET and ¹H-MRS), morphological (DWI), and anatomical end-points (MRI) for mTOR and HSP inhibitor response in mouse pancreatic adenocarcinoma xenografts.

Methods: Human Panc-159, patient-derived pancreas adenocarcinomas, were established as heterotopic xenografts in athymic nude mice. Study drugs were administered as follows: daily 5mg/kg of everolimus via oral gavage and/or weekly 100 mg/kg of ganetespiib via tail vein injection. PET and DWI studies were performed at baseline, mid-study (Day 8-9), and end of study (Day 21-22). For PET studies, 150 µCi of FDG was injected via tail vein. After 1-hr uptake, 10 minute emission was acquired with an Inveon Siemens micro PET/CT scanner. Axial images were analyzed by AsiProVM to determine metabolic activity determined as standardized uptake values (SUV). For MRI, animals were placed into a 4.7 Tesla Bruker PharmaScan MRI with a 31mm-diameter Bruker volume coil. RARE PD, to assess volume, parameters were as follows: FOV = 4cm; slice thickness = 1.2mm; TE/TR = 4000/31.9ms; number of slices = 16; number of averages = 2; matrix size = 180 degrees; total acquisition time = 4.1 minutes; b-values = 256x256. DWI parameters were as follows: FOV = 4.6cm; slice thickness = 2 mm; TE/TR = 3000/40ms; number of slices = 4; number of averages = 16; matrix size = 64x64; total acquisition time = 15 minutes; b-values = 0, 150, 300, 600, 800, 1000 s/mm². All images were analyzed with Bruker Paravision 3.0.2 software. At end of experiment, all mice were sacrificed, and tumors were harvested and extracted with perchloric acid. All ex vivo ¹H-MRS on hydrophilic and lipophilic extracts were obtained on a 400MHz Bruker Avance spectrometer using a 5 mm-BBI probe, and multivariate analysis (3D-component partial least square linear discriminate analysis, PLS-LDA) was conducted with MetaboAnalyst2.0 software.

Results:

Figure 1: Representative Day 21-22 tumor images. (a, d): RARE PD images. (b, e): DW images; average ADC value of tumors are shown on figure. (c, f): FDG-PET images; arrows indicate tumor.

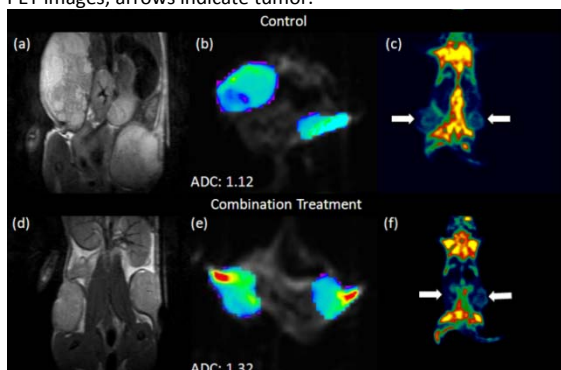


Figure 2: PLS-LDA multivariate analysis. Variables with highest importance in projection (VIP): **ADC** (3.0262), **tumor volume** (2.3178), **glycerol-PI** (1.9073), **FDG-SUV** (1.3406).

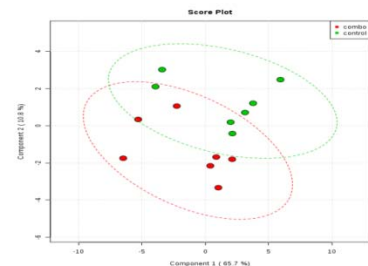


Table 1		Day 8-9		Day 21-22	
		Fold Change	P-value	Fold Change	P-value
Volume	Control	2.09	0.127	4.42	0.054
	Combination	1.47	0.006	2.76	0.0018
	Everolimus	2.21	0.320	4.76	0.427
	Ganetespiib	1.77	0.144	3.30	0.100
FDG-SUV	Control	1.37	0.173	1.19	0.341
	Combination	0.573	0.055	0.351	0.0085
	Everolimus	0.456	0.060	0.190	0.0296
	Ganetespiib	0.938	0.121	0.565	0.0597
ADC	Control	0.908	0.278	0.948	0.359
	Combination	1.10	0.195	1.23	0.0323
	Everolimus	1.01	0.431	1.12	0.0813
	Ganetespiib	0.957	0.119	0.926	0.159

Single treatments of ganetespiib or everolimus did not show inhibition of tumor growth nor decrease in tumor cellularity (Table 1). Although, it should be noted that everolimus treatment did cause a slight decrease in glucose uptake (as previously reported for mTOR inhibition), while ganetespiib did not change glucose uptake. Meanwhile, Table 1 shows that the combination treatment not only caused an inhibition of tumor growth but also a decrease in tumor cellularity (increased ADC values). In addition, the combination treatment also showed inhibition of tumor metabolic activity as demonstrated through the decrease in glucose uptake. Figure 1 shows the representative trends of the combination treatment resulting in decreased tumor volume size (d), decreased cellularity (e), and decreased glucose uptake (f) compared to control. Multivariate PLS-DA analysis on quantitative data sets (including tumor volume, FDG-SUV, ADC, and ¹H-MRS derived concentrations of endogenous metabolites) shows good group separation between the control and combination treatment groups (Figure 2). ADC values, tumor volumes, phospholipid-associated glycerol (glycerol-PL), and FDG-SUV were the most important variables accounting for group separation (highest VIP scores).

Conclusions: Our study provides the rationale of combining two STIs, everolimus (an mTOR inhibitor) with ganetespiib (an HSP-90 inhibitor) to overcome drug resistance and increase treatment responsiveness in pancreatic cancer. While not completely cytostatic, the combination treatment did result in slowing down of tumor growth and significant decrease in cellularity, phospholipid metabolism and glucose uptake giving potential for these variables to serve as effective imaging biomarkers for therapeutic response. Even though Panc-159 is highly resistant to single everolimus treatment (mTOR inhibition alone), this combination with an HSP-90 inhibitor provides the first evidence of proliferation and metabolic response by functional multi-parametric imaging and FDG-PET, DWI and ¹H-MRS should be considered as potential biomarkers in clinical trials.