Treatment with the novel PI3K inhibitor PX-866 results in ^{1}H MRS detectable changes in a glioma model *in vivo*

S. M. Ronen[1], J. Banskon[2], D. Koul[3], M. Sankaranarayanapillai[1], D. Webb[2], C. Kingsley[2], J. Delacerda[2], J. Edge[3], A. Yung[3], and G. Powis[4]

[1]Experimental Diagnostic Imaging, U.T. M.D. Anderson Cancer Center, Houston, Texas, United States, [2]Imaging Physics, U.T. M.D. Anderson Cancer Center, Houston, Texas, United States, [3]Neuro-Oncology, U.T. M.D. Anderson Cancer Center, Houston, Texas, United States, [4]Experimental Therapeutics, U.T. M.D. Anderson Cancer, Houston, Texas, United States

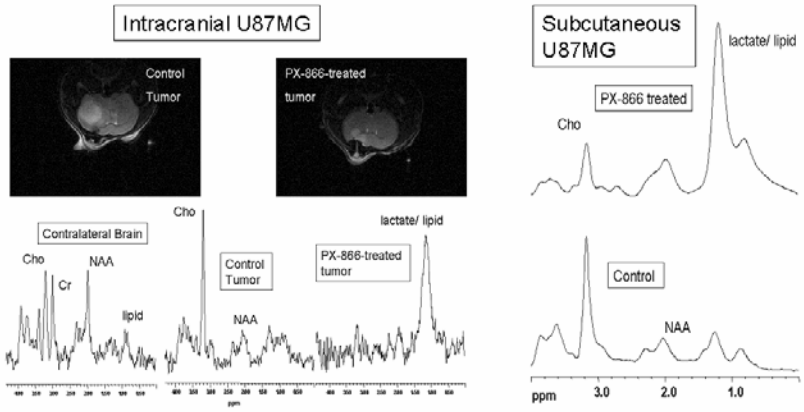
Introduction. Novel cancer therapeutics that are in development and in clinical trials increasingly target known oncogenic mutations. One such drug is the small molecule PI-3-kinase (PI3K) inhibitor PX-866, a semi-synthetic viridin analogue of wortmannin. (1). PX-866 is a biologically stable inhibitor that specifically antagonizes the p110-α, β and γ subunits of PI3K and induces prolonged inhibition of tumor PI3K signaling following both oral and intravenous administration. Thus**,** PX-866 will be entering clinical trials at our institution. Our goal is to develop noninvasive pharmakodynamic markers of drug molecular action. We have previously shown (2) that inhibition of PI3K with wortmanin, as well as another PI3K inhibitor - LY294002, results in detectable changes in the MR spectra of different cancer cell lines. Here we show that MRS can be used to monitor molecular response to PX-866 in a glioma model *in vivo*.

Materials & Methods Both intracranial and subcutaneous U87MG glioma tumors (3) were investigated. For the orthotopic model, tumor cells were implanted using non-ferromagnetic screw-guides 1 mm anterior and 1.2 mm lateral to the bregma and $5x10^5$ U87MG glioma cells in 10 µl PBS were injected in the region of the putamen. For subcutaneous tumors, $3x10^6$ cells were implanted in the flank area. Treatment with PX-866 (2mg/Kg po – treated animals), or with carrier PBS (control animals) began on day 4 after cell inoculation. Animals were treated daily for 15 days in the case of the intracranial tumors and for 28 days in the case of the subcutaneous tumors. All spectroscopic and imaging measurements were conducted using a 7.0T Biospec USR imaging system (Bruker Biospin). A linear volume resonator with 72 mm inner diameter (ID) was used for signal excitation in mini-imaging gradients (116 mm ID). An electronically tuned, actively decoupled custom RF coil (13 mm ID) was used for signal detection. A 3-plane RARE imaging sequence was used to confirm positioning, and axial and sagittal multi-slice T_2-weighted images (TE/TR 60ms/3000ms, RARE factor 8, 4 averages, 156µm x 156µm x 1mm resolution) were collected for tumor visualization. Following automatic and manual shim adjustment, point-resolved spectroscopic (PRESS) measurements (TE/TR 20ms/2500ms, 4kHz bandwidth, 200 averages, 2048 points) with and without water suppression were acquired over cuboid volumes ranging from 1.5 mm^3 to 80 mm^3 depending on tumor size. Care was taken to localize the voxel inside the tumor volume only. Individual peak area were determined by deconvolution and normalized to the water signal acquired from the same voxel.

Results & Discussion In the case of intracranial tumors (figure, left), tumor volumes dropped from 22±13 mm^3 in control animals (n=5) to 7±5 mm^3 in PX-866 treated animals (n=5, p=0.06). As expected, spectra of untreated tumors and normal contralateral brain differed significantly. The average total choline to NAA ratio (Cho/NAA) was 0.75±0.08 in contralateral brain versus 1.8±0.3 (p<0.002) in the gliomas. Following treatment, average Cho/NAA dropped 30% to 1.2±0.6 in treated tumors, while the spectrum from contralateral brain remained unchanged. However, large variations were observed between animals. Thus, only the smaller treated tumors (n=2) exhibited Cho/NAA values comparable to controls, while the spectrum of larger tumors was affected little. Interestingly, the smaller tumors also exhibited a substantial increase in the lipid/lactate peak (see figure), typically associated with necrosis. The effects of treatment could not be observed using either DCE-MRI or diffusion weighted imaging (data not shown).

In the case of the subcutaneous gliomas, PX-866 treatment produced no significant effect on tumor size which was ~200 mm^3 on average for both control and treated groups. Nonetheless, the spectra obtained from control and PX-866-treated tumors (figure, right) differed significantly demonstrating a drop in choline, an increase in NAA and a large increase in lactate/lipid levels, thus reproducing the spectral changes observed in the smaller treated intracranial tumors. Specifically, Cho/NAA dropped significantly from 2.1±0.3 (n=3) to 0.5±0.5 (n=5, p<0.002) following PX-866 treatment and the Cho to lipid ratio dropped from 1.3±0.3 to 0.2±0.2 (p<0.02).

Our findings demonstrate the potential of MRS to monitor the molecular effect of PX-866 in gliomas in vivo.



Left: Control and PX-866 treated intracranial U87MG tumors. Top: T2 weighted images. Bottom: ^{1}H spectra from contralateral brain, untreated (control) tumors, and treated tumors.

Right: ^{1}H spectra from control and treated subcutaneous U87MG tumors.

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