Comparison of FDG-PET and Hyperpolarized Pyruvate in Assessing Response to an Isoform-specific PI3K inhibitor in Breast Cancer

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Target audience: Researchers and clinicians interested in PI3K inhibition and hyperpolarized ^{13}C for monitoring response to therapy in cancer.

Introduction: Phosphoinositide 3-Kinase (PI3K) has been shown to modulate multiple steps in glucose uptake and metabolism through activation of the protein kinase, AKT. In order to dissect the contributions of PI3K-pathway components, we examined the effects of an isoform-specific PI3K inhibitor on glycolysis using FDG-PET and hyperpolarized MRI. PI3K possesses multiple isoforms that are differentiated by their structure and substrate affinity. Here we compare the effects of a PI3Kα–specific inhibitor (NVP-BYL719 [1]) on FDG-PET and hyperpolarized ^{13}C spectroscopy in a mouse model of breast cancer. The results indicate that hyperpolarized pyruvate is more sensitive than FDG-PET in detecting metabolic effects of this inhibitor, and that hyperpolarization and FDG-PET can play complimentary roles in assessing the effects of PI3K inhibitors on glycolysis.

Materials and Methods: Experiments were conducted with approval from the Institutional Animal Case and Use Committee. A model for BRCA1-related breast cancer was generated by transplantation of spontaneously developed tumor tissue from a K14-Cre BRCA1f/f p53f/f donor to the mammary fat pads of K14-Cre negative littermates. Tumors were then permitted to grow to 10mm diameter. Eight mice were divided into two groups (n=4 each for FDG-PET and hyperpolarized ^{13}C). Each mouse was imaged twice, at baseline and after treatment. Prior to the second scan each mouse received two doses of NVP-BYL719 (30mg/kg by gavage) 2 and 12 hours before imaging. For PET studies, 0.3-0.4 mCi of FDG was administered retro-orbitally in anesthetized mice one hour prior to imaging with a NanoPET/CT (Bioscan/Mediso) scanner. A whole-body FDG-PET scan was acquired and counts per minute were normalized for region-of-interest volume and dose. To correct for metabolic variability between exams and determine tumor uptake changes, FDG-uptake rates were corrected for cardiac FDG uptake. For MRI studies, mice were anesthetized using isoflurane in oxygen, and a 30G needle connected to an extension tube was placed in the tail vein. Anesthetized mice were placed in a 9.4T scanner (Biospec, Bruker, Billerica, MA), where respiration was maintained at approximately 70/minute and body core temperature was maintained at 37C. A 28mm diameter ^{13}C surface coil was placed around each tumor. After acquisition of T2-weighted proton images and local shimming within the tumor to obtain a proton linewidth of 120Hz or less, a slice was prescribed covering as much as possible of the tumor while excluding neighboring normal tissues. This was possible because the tumors in this model are relatively superficial (Fig. 1). Hyperpolarized ^{13}C pyruvate solution was prepared using standard methods in a DNP polarizer (Hypersense, Oxford Instruments, Oxfordshire UK). 250µl of 100mM pyruvate solution were administered by tail vein during acquisition of 64 slice-selective ^{13}C spectra from the tumor (7.5-degree tip angle, 2s TR, 6.5kHz spectral width and 8192 spectral points). Spectral lines from pyruvate and lactate were integrated using a time-domain fitting method (AMARES in jMRUI [2]). Signals from pyruvate and lactate were then summed over the 64 scans to compute the time integral of each metabolite's signal. The ratio of the lactate integral to the pyruvate integral was then computed [3] as a measure of the rate of lactate formation and ^{13}C label exchange.

Results and Discussion: Fig. 2 shows representative slice-selective spectra and metabolite time courses acquired within a tumor before and after administration of NVP-BYL719. The unusual appearance of two maxima in the pyruvate time course was observed in several animals of this type and may suggest complex vascular delivery of the agent in this model. Fig. 3 summarizes the percentage

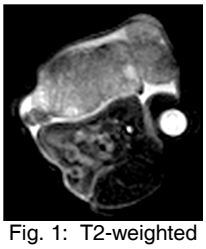

Fig. 1: T2-weighted proton image of a representative tumor.

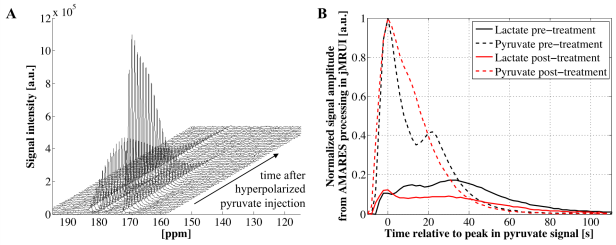

Fig. 2: Spectra (left) and pyruvate and lactate time courses (right) in a representative tumor.

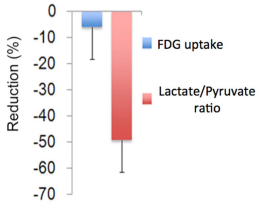

Fig. 3: Reductions in FDG uptake and lactate/pyruvate ratio after treatment (see text).

change in FDG uptake and lactate/pyruvate ratio in the two groups after NVP-BYL719 treatment. While FDG-PET showed a minor, non-significant reduction in uptake, the area-under-the-curve analysis of the pyruvate data showed a 34-62% reduction in lactate relative to pyruvate that was significant (p=0.01, two-tailed Student's t-test). By contrast, non-specific inhibition of PI3K has been previously shown to yield larger (~40-90%) reductions in FDG uptake [4].

Conclusions and Acknowledgement: These data indicate that hyperpolarized pyruvate is more sensitive than FDG-PET in assessing the metabolic effects of PI3Kα inhibition, and that the effects of PI3Kα inhibition on glycolysis may occur downstream of hexokinase. This work was supported in part by the NIH through grants R21 EB014471 and R01 CA169470.

References 1. P. Furet *et al*., *Bioorg Med Chem Lett* **23**, 3741. 2. Naressi et al. Magn Reson Mater Phys (2001) 12:141-152. 3. Hill, D. K., Y. Jamin, *et al.* NMR Biomed (2013) 26: 1321-1325. 4. A Juvekar *et al*., Cancer Discovery (2012) 2:1048.