

# Distinguishing Sensitive and Resistant Early Therapy Response of Pancreatic Tumor Xenografts Using $^{13}\text{C}$ -MRS of Hyperpolarized Pyruvate

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## Background:

Hyperpolarized  $^{13}\text{C}$  MRS/I using the dissolution DNP (dynamic nuclear polarization) method provides a >10,000 fold signal enhancement for detecting  $^{13}\text{C}$  labeled probes of endogenous, nontoxic, substances such as pyruvate to monitor metabolic flux through multiple key biochemical pathways (glycolysis, citric acid cycle and fatty acid synthesis). The recent technological advancement has permitted noninvasive real-time imaging and spectroscopy of tumor cell metabolism in vivo following intravenous administration of  $^{13}\text{C}$ -labeled cell substrates [1]. The Warburg effect is a metabolic feature of cancers that causes them to preferentially metabolize pyruvate via glycolytic pathway to lactate [2]. It is reported that the inhibition of LDH-A (lactate dehydrogenase-A) with the small molecule drug FX11 inhibits tumor progression [3]. In this work, we have evaluated the efficacy of FX11 treatment to human pancreatic xenograft tumors via assessing the metabolic conversion of lactate from pyruvate using  $^{13}\text{C}$  magnetic resonance spectroscopy.

## Method:

Hyperpolarization experiments were performed with 1- $^{13}\text{C}$ -labeled pyruvic acid containing 15 mM trityl radical (OX63) for one hour using a DNP polarizer (HyperSense). Hyperpolarized pyruvate was dissolved in dissolution media containing NaOH, NaCl and EDTA (ethylenediaminetetraacetic acid) to bring the sample with average physiological pH of 7.6. 350  $\mu\text{L}$  hyperpolarized pyruvate after dissolution was administered via a jugular-vein catheter to tumor bearing mice in 15-18 sec.  $^{13}\text{C}$ -spectra were obtained using a Agilent 7 T imaging scanner utilizing a dual tuned  $^1\text{H}$  -  $^{13}\text{C}$  volume coil. Fresh pancreatic cancer tissues, excised from patients at Johns Hopkins Hospital (pancreatoduodenectomy), were transplanted as subcutaneous tumors in 6-week-old athymic nude mice.

## Results and Discussion:

Two set of tumors that are sensitive (JH015, Panc253) and resistant (JH024, JH033) to FX11 have been chosen for hyperpolarized  $^{13}\text{C}$ -MRS study. Data acquisition was initiated right before the hyperpolarized pyruvate injection with a  $T_R$  of 1 sec and flip angle of  $9^\circ$ . In-vivo dynamic MR spectra show peaks for pyruvate and its metabolic conversion to lactate via LDH activity (Fig. 1). The tumors were treated with FX11 for seven days with doses of 42  $\mu\text{g}$  daily through i.p. injection, whereas the control group received DMSO. The Lac/Pyr ratio was calculated and has been used as FX11 response marker before and after treatment and control group as well. In the drug-sensitive JH015 and Panc253 tumors, the Lac/Pyr ratios were significantly lower in treated animals relative to DMSO controls ( $p < 0.01$ ), suggesting drug response (Fig. 2). In drug-resistant JH024 and JH033 tumors, no significant differences relative to controls were observed. Differences in Lac/Pyr ratios were observed much sooner than were differences in tumor volume. Ki-67 and TUNEL staining were performed on post-treatment tumor specimens to determine the effect of FX11 on apoptosis and tumor cell proliferation. Our experimental observation using  $^{13}\text{C}$ -MRS on FX11 sensitive and resistant tumors has been validated through the other imaging modalities such as FDG-PET and diffusion weighted MRI (DW-MRI) measurements of the apparent diffusion coefficient of tissue water (ADC). In FX11 sensitive tumors, the FDG uptake reduced compared to control group. More importantly, the ADC increased in treated sensitive tumors but resistant tumor ADC decreased. All these results will be presented.

## Conclusion:

Hyperpolarized  $^{13}\text{C}$ -MRS/I is a potential biomarker that informs on specific metabolic pathways associated with disease progression and response to therapy. Measurement of hyperpolarized  $^{13}\text{C}$  label flux between pyruvate and lactate is used to detect early response to therapy before differences in tumor volume were observed.

## References:

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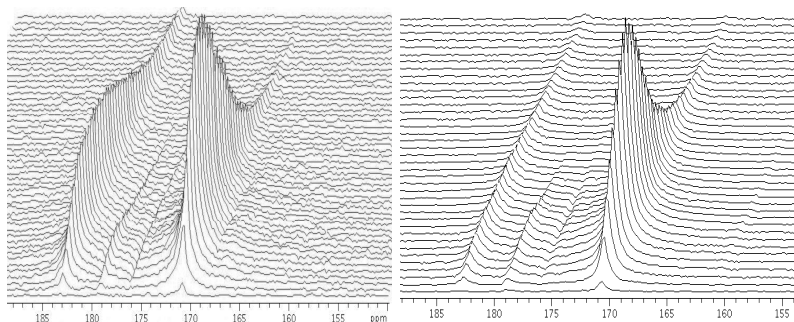


Fig.1. Dynamic in-vivo  $^{13}\text{C}$  MR spectra in untreated tumor (Panc253). (Pyruvate & lactate peaks are at 171 & 183 ppm respectively. The relative ratio of Lactate to Pyruvate (Lac/Pyr) is higher (left). After six days of treatment with FX11, the Lac/Pyr ratio reduced significantly (right).

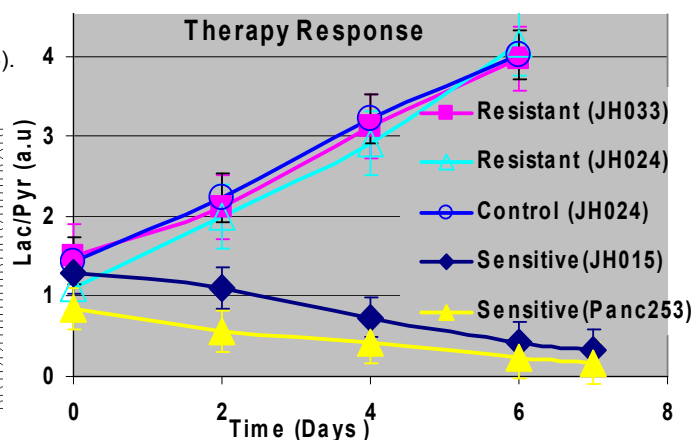


Fig. 2. Response marker (Lac/Pyr ratio) was reduced with FX11 treatment in sensitive tumors and increased in resistant tumors. In control (DMSO treatment) group, the Lac/Pyr ratio is also increased.