Effect of PKM2 Activator and 2-Deoxyglucose Treatments on Cancer Metabolism Measured in vivo by hyperpolarized 13C MR Spectroscopic Imaging

Jae Mo Park[1], Sui-Seng Tee[1], Ralph Hurd[2], Kyle Brimacombe[3], Matthew Boxer[3], Dirk Mayer[4], Brian Rutt[1], and Daniel Spielman[1]

[1]Radiology, Stanford University, Stanford, CA, United States, [2]GE Healthcare, Menlo Park, CA, United States, [3]National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, United States, [4]Diagnostic Radiology and Nuclear Medicine, University of Maryland, MD, United States

Target Audience Researchers and clinicians who are interested in anti-cancer treatment strategy.

Purpose Metabolic reprogramming of tumor-specific metabolism to normal state has been tried as an alternative therapeutic strategy for anti-cancer treatment. For instance, elevated glucose metabolism and lactate (Lac) fermentation (Warburg effect), one of the most well-known metabolic phenotypes of tumor metabolism, has been received much attention. A recent study showed that small molecule activators of PKM2, TEPP46, inhibit xenograft tumor growth[1]. PKM2, the M2 isoform of pyruvate (Pyr) kinase, plays a role in the last step of glycolysis, converting phosphoenolpyruvate (PEP) to Pyr. So far, PKM2 is expressed in all tested cancer cells[1]. PKM2 exists either as a dimer or a more active, tetrameric form. The less active dimer is thought to favor rapid cell division by the diversion of glycolytic intermediates into biosynthetic pathways[2]. More recently, Tee et al. hypothesized that the use of a glucose analogue, 2-deoxyglucose (2DG), in combination with the PKM2 activator will accelerate the uptake of the toxic 2DG in tumors[3], and showed that the combination therapy was indeed more effective than single treatment of TEPP46 or 2DG in breast cancer cells. Another study[4] of PKM2 treatment on lung cancer cells showed a metabolic difference in treated cells relative to vehicle-treated controls using hyperpolarized ^{13}C MR spectroscopy, which enables a real-time investigation of metabolic kinetics, suggesting that ^{13}C-labeled Lac-to-Pyr ratio might be a biomarker to measure the treatment effect. In this study, we applied the PKM2-2DG treatment to tumor-bearing mice and observed the therapeutic response in Pyr metabolism using hyperpolarized ^{13}C Pyr MR spectroscopic imaging (MRSI).

Methods Approximately 5x10^6 H1299 lung cancer cells were injected into mice subcutaneously 8-12 weeks before the imaging study. The progression of the tumors was volumetrically monitored daily to achieve comparable tumor sizes (~500 mm^3). All mice (n=9) were imaged with hyperpolarized ^{13}C MRSI in three groups: 2DG- (n=2, 60 mg/kg body weight), TEPP46- (n=3, 1 mg/kg body weight), and combination-treated (n=4). Animals were anesthetized with 1-1.5 % isoflurane in oxygen (~1.5 L/min) and a catheterized in a tail vein before being placed at the center of a clinical GE 3T MR scanner and a high performance insert gradient coil (G_{max}=500mT/m, Slew rate$_{max}$=1865 mT/m/ms). A custom-made ^{13}C-^{1}H dual-channel coil was used for both RF excitation and signal acquisition. Vital signs were monitored throughout the experiments, and the body temperature was maintained ~36.5 ^{o}C using a temperature-regulated air heater. The homogeneity of the B_0 field over the tumor region was manually optimized with a point-resolved spectroscopy sequence using the linear shim currents. Single-shot spiral chemical shift imaging (CSI) with spectral undersampling (field of view = 43.5x43.5 mm^2, 6mm slick thickness, matrix size = 16x16, variable flip angle RF excitation, temporal resolution = 3s, 16 time points, 32 echoes, spectral bandwidth = 280 Hz) was used for dynamic metabolic imaging of hyperpolarized [1-^{13}C]Pyr and its products[5,6]. The ^{13}C spectra were acquired 6s after the start of a bolus injection (300 μL of 80mM Pyr over ~20s) at baseline and 2 hr after the treatments. For anatomical references, both T_2-weighted fast spin-echo and contrast-enhanced T_1-weighted spin-echo images were acquired. Integrated signal for individual metabolite peak in time-averaged spectrum was used to calculate Lac-to-Pyr ratios. Paired student t-test was performed to evaluate statistical significance of treatment effects.

Results and Discussion Lac/Pyr ratios in tumors had a large variation between animals probably due to the discrepancy in blood vessel development and perfusion. Nonetheless, Lac/Pyr consistently increased in all mice when they were treated with TEPP46 and 2DG (0.27±0.12 at baseline to 0.43±0.09 at 2hr post-treatment, P<0.05), whereas no significant change in the ratio was observed neither 2DG-only (P>0.2) nor PKM2-only (P>0.2) treated groups. The increase in the ratio suggests that there might be a synergic anti-cancer mechanism of the PKM2 activator and 2DG. The interpretation for Lac/Pyr increase in the dual-treated group could be controversial, but it is possibly because tumors look for any energy source while upregulated 2DG uptake in the tumor cuts off glucose availability as an inhibitory substitute. Therefore, the injected [1-^{13}C]Pyr bolus could be used as a primary source in the tumor, leading to more Lac production.

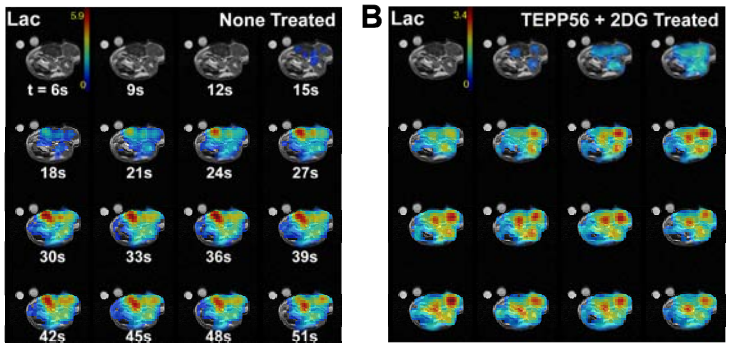

Fig.1 Dynamic Lac maps before (A) and 2hr after (B) TEPP56+2DG.

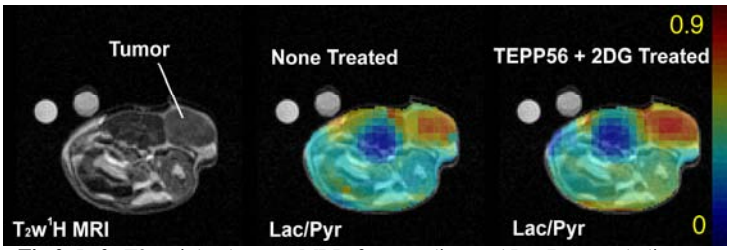

Fig.2 (Left) T2-weighted proton MRI of tumor slice, and Lac/Pyr metabolite maps before (middle) and 2hr after (right) TEPP46+2DG.

Conclusion The TEPP46-2DG combination therapy showed metabolic perturbation in tumors *in vivo*, suggesting a potential therapeutic value of the treatment strategy. Lac/Pyr ratio in tumors after an injection ^{13}C-Pyr might serve as a precursor to assess the treatment effect, or possibly a measure for the degree of glucose starvation in tumors.

References **1.** Anastasiou D et al, *Nat Chem Biol.* 2012; 8(10):839-47, **2.** Mazurek S et al, *Int J Biochem Cell Biol* 2011; 43(7):969-80, **3.** Tee S et al, *Int Conf Mol Tar Cancer Therap* (AACR) 2013, **4.** Tee S et al, *WMIC.* 2013, **5.** Mayer D et al, *Magn Reson Med.* 2011; 65(5):1228-33, **6.** Park JM et al, *Magn Reson Med.* 2012; 68(6):1886-93.

Acknowledgements NIH: EB009070, AA005965, AA0018681, AA13521-INIA, P41 EB015891, DOD: PC100427, Lucas Foundation, and GE Healthcare