# Assessment of Prostate Cancer Aggressiveness with Hyperpolarized Dual-Agent 3D Dynamic Imaging of Metabolism and Perfusion

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**Target Audience**: Researchers interested in prostate cancer and hyperpolarized $^{13}$C imaging.

**Purpose:** Prostate cancer is the second deadliest type of cancer in men in 2014, and imposes a great burden on US healthcare[1]. A major challenge in the clinical management of prostate cancer is the differentiation of aggressive cancer from indolent disease. To address this unmet need, this project was designed to detect both the increased LDH activity and abnormal vasculature in aggressive prostate cancers[2]. This study applied hyperpolarized (HP) $^{13}$C-pyruvate & $^{13}$C-urea dynamic EPSI to simultaneously measure metabolism and perfusion kinetics in transgenic mouse model of prostate cancer (TRAMP)[3,4]. Pathological, immunohistochemical and gene expression analyses were performed following HP MRI in order to separate mice having low and high grade prostate cancer and to understand the interplay between perfusion and metabolism in aggressive (high grade) prostate cancer.

**Methods:** Sequence: A 3D dynamic compressed-sensing $^{13}$C-EPSI sequence was developed for simultaneous imaging of metabolism ([1-$^{13}$C]pyruvate) and perfusion ([1-$^{13}$C]urea) covering the entire mouse with high spatiotemporal resolution (spatial resolution 3.3 x 3.3 x5.4mm, 2s temporal) while ensuring optimal SNR for kinetic modeling. Specialized multiband excitation pulses were used with variable flip angles designed using the T1-effective scheme[5,6].

Metabolic Modeling: Quantitative estimation of metabolic metrics was done using a compartmental model, as described previously[7]. Metabolic and perfusion dynamics were assessed independently.

Improved Perfusion Modeling: It is known that timing errors in the $^{13}$C perfusion curve[8] can have substantial impact on non-linear fitting of the perfusion kinetics. In this study we applied an improved joint fitting scheme utilizing both the perfusion curve and arterial input function (AIF) as input from the whole-animal coverage of the 3D dynamic datasets. The AIF was corrected for the intra-voxel partial volume effect. Sensitivity analysis on timing errors revealed that a typical 2-second offset in the tumor perfusion curve creates about 15% difference in perfusion index estimates which were small relative to the differences detected between low and high grade tumors.

Hyperpolarization and Imaging: [1-$^{13}$C] pyruvate and [$^{13}$C] urea were co-polarized for 2 hours using a 3.35T prototype GE SpinLab polarizer[9]. The co-polarized $^{13}$C biomarkers were injected over 15 seconds through a tail vein catheter, and the 3D dynamic acquisition was initiated immediately at the end of injection. Imaging was done on a clinical GE 3T scanner using a dual-tuned mouse coil.

Histochemical and gene expression analysis: Blocks of tumor tissue excised upon sacrifice were immediately fixed with 10% formalin or frozen individually. Fixed tissue was sliced into 5um slabs and processed using standard H&E and other immunostaining protocols. The histochemical samples were graded based on the cellularity and the percentage of well and poorly differentiated prostate cancer. Tissues were also analyzed for proliferation (Ki-67) and hypoxia (PIM). RNA was extracted from frozen tissue and subjected to RT-PCR amplification for the expression of key enzymes using standard qPCR protocol[10].

**Results:** 6 of the 11 TRAMP tumors studied were categorized as high grade based on pathology (high versus low grade - 95% ± 9 % versus 40 ± 8% poorly differentiated cells, respectively). Rates of pyruvate to lactate conversion ($k_{pl,late}$ = 0.0509 ± 0.0145 vs $k_{pl,early}$ = 0.0189 ± 0.0025) were significantly higher (P<0.001) in high-grade tumors, based on the modeled analysis of HP-$^{13}$C MRSI dynamic data. These high grade cases also demonstrated a 2-fold higher rate of proliferation (fig. 1D - Ki-67). Perfusion as measured by HP $^{13}$C urea was significantly (P<0.003) reduced in high- relative to low-grade tumors. Poor perfusion resulted in increased hypoxia (fig. 1D - PIM staining) leading to increased HIF1A expression and a further increase in LDHA and reduction in LDHB expression relative to early stage tumors. The 3 fold increase in $K_{pl}$ in high grade TRAMP tumors correlated well with the 2 fold increase in LDH activity combined with the increase in LDH-5 isoform.

**Conclusions:** We developed a novel imaging protocol to quantitatively assess aggressive prostate cancer on transgenic mouse models using dual agent HP $^{13}$C-pyruvate & $^{13}$C-urea dynamic 3D metabolic and perfusion MR. Simultaneous imaging of metabolism and perfusion dynamics demonstrated a significant correlation of hyperpolarized lactate production rates with histological grade. Also the significantly reduced perfusion observed in high grade tumors supports the known hypoxia, resulting in further increased hyperpolarized lactate production.

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**References:** [1] ACS. 2014. [2] Nelson et al. JMR. 2010. [3] Siriam et al. in press. 2014. [4] Hurwitz et al. C P Immunology. 2001. [5] Xing et al. JMR. 2013. [6] Larson et al. MRM. 2011. [7] Chen et al. ISMRM. 2014. [8] von Morze et al. MRM. 2013. [9] Hu et al. MRM. 2012. [10] Kurhanewicz et al. Neoplasia. 2012.

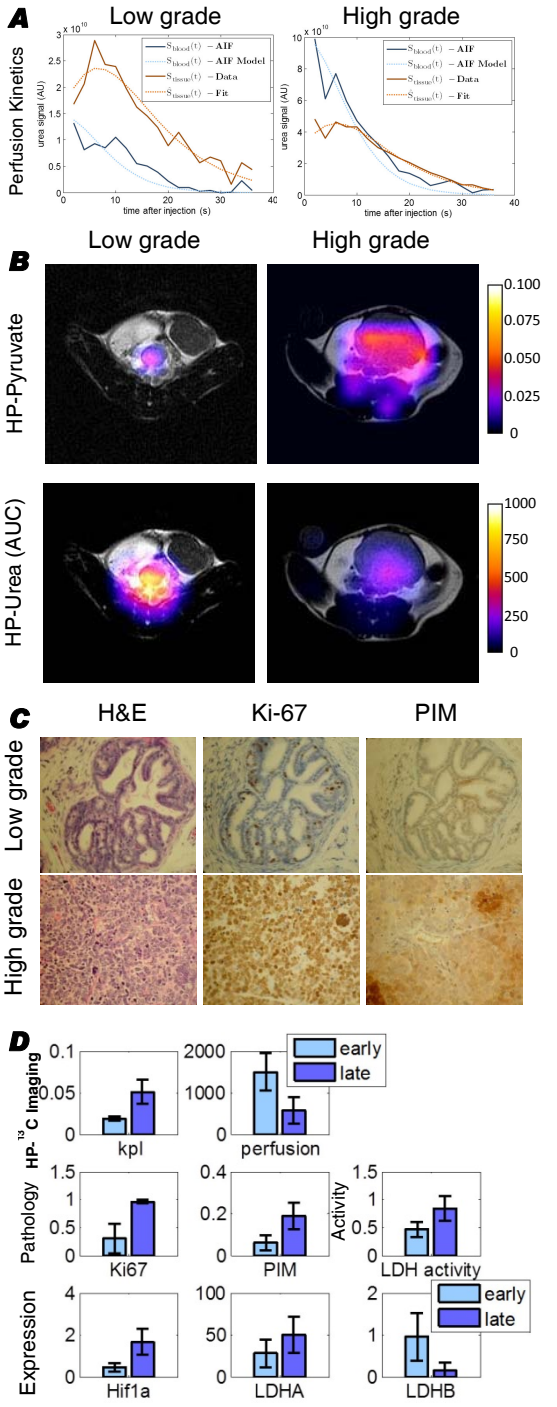


**Fig 1** **A.** Example showing the modeling of perfusion kinetics using joint fitting of the urea AIF and tumor tissue dynamics in both high and low grade TRAMP tumors. **B**. kpl and perfusion images overlaid on $T_2$-FSE reference images showing increased metabolism and reduced perfusion in high- versus low-grade TRAMP tumors. **C**. Representative H&E, Ki-67 and PIM stained high and low grade TRAMP tumor sections. D. (top) Bar plot summarizing Kpl and perfusion (urea AUC) in high- versus low-grade tumors (N=11), (middle) comparison of % KI-67, PIM staining and LDH activity from the same tumors, (bottom) expression of key enzyme changes that contribute to the increase in aerobic glycolysis in high grade tumors.