

# Use of hyperpolarized $^{13}\text{C}$ -MRS to monitor tumor response to Sorafenib treatment, in comparison with diffusion weighted-MRI.

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**Target audience:** MR scientist with interest in the monitoring of the response to anti-tumoral treatment.

**Purpose:** Sorafenib is a multikinase inhibitor approved for human use in cancer. Conventional anatomically based endpoints may be inadequate to monitor the tumor response to targeted agents that usually do not result in tumor shrinkage while used in monotherapy. Therefore, the identification and use of more appropriate, non invasive biomarkers are needed to optimize the schedule and dosage of novel therapeutic approaches.  $^{13}\text{C}$  magnetic resonance spectroscopy (MRS)-detectable hyperpolarized fumarate to malate conversion has been validated as a marker of cell death and treatment response in tumors (1). In addition,  $^{13}\text{C}$  magnetic resonance spectroscopy (MRS)-detectable hyperpolarized (HP) pyruvate to lactate conversion has recently been suggested as a marker of response to a MAPK inhibitor (2). The aim of the current study was to assess the response to a kinase inhibitor, Sorafenib, using a combined HP  $^{13}\text{C}$ -fumarate and  $^{13}\text{C}$ -pyruvate study in mammary xenografts, in comparison with diffusion weighted-MRI (DW-MRI) and histological markers.

**Methods:** Mice bearing MDA-MB-231 xenografts, were imaged at day 0, day 2, and day 5 of daily intraperitoneal treatment with 40mg/kg of Sorafenib.  $[1,4-^{13}\text{C}_2]$  fumaric acid (FA) or  $[1-^{13}\text{C}]$  pyruvic acid (PA) were mixed with 15mM trityl radical (OX63) and hyperpolarized by an Oxford DNP Polarizer, HyperSense®. The polarized substrate was quickly dissolved in Tris/EDTA, NaCl and NaOH at 37°C, yielding 20mM FA or 80mM PA at neutral pH, before injection to the mouse via jugular vein catheter (0.45ml FA and 0.35ml PA). Two injections were made in 1H apart. Mice were imaged using a double tuned  $^1\text{H}$ - $^{13}\text{C}$  volume coil in an Agilent ASR310 7T. Hyperpolarized  $^{13}\text{C}$  FA and  $^{13}\text{C}$  PA spectra were acquired during 5 minutes with specific  $T_R$  (FA:2000ms and PA:1000ms) and flip angle (FA:15° and PA:9°) from a 4mm thick slice across the tumor. DW-MRI was performed before the injection using a spin-echo sequence, (3 b-values,  $T_R$ :1500ms, TE:36ms). Tumors were taken at day 5 for histological analysis and compared to untreated tumors.

**Results:** Daily sorafenib injections for 9 days were able to significantly reduce MDA-MB-231 tumor growth. Malate to fumarate (MA/FA) ratio was progressively increased from day 2 until day 5 (Fig.1A and Fig.2), similarly to the changes observed in ADCw (Fig.1B). A positive correlation was established between the relative change in MA/FA and the relative change in ADCw over time (Fig.1C). No significant change was observed in the lactate to pyruvate ratio over time during treatment with Sorafenib (Fig.1D). Tumor size did not change significantly between day 0 and day 5 under Sorafenib treatment, whereas H&E histological analysis did show a significant increase in tumor necrosis (40.7% increase) between untreated and Sorafenib treated tumors (for 5 days).

**Discussion and conclusion:** Hyperpolarized MRS using  $^{13}\text{C}$ -fumarate is showed to be an early in vivo marker of response to Sorafenib and is positively correlated with DW-MRI, with a higher sensitivity for  $^{13}\text{C}$ -fumarate with respect to DW-MRI (2.8 vs 1.3 respectively at day 5). Results are in accordance with ex vivo H&E analysis. The lactate to pyruvate ratio does not seem to be an in vivo marker of tumor response to the MAPK inhibitor, contrarily to in vitro studies using U0126.

**References:**

- Gallagher FA, Kettunen MI, Hu DE et al. Production of hyperpolarized  $[1,4-^{13}\text{C}_2]$ malate from  $[1,4-^{13}\text{C}_2]$ fumarate is a marker of cell necrosis and treatment response in tumors. Proc Natl Acad Sci. 2009;106(47): 19801–19806
- Lodi A., Woods S.M.and Ronen S.M.Treatment with the MEK inhibitor U0126 induces decreased hyperpolarized pyruvate to lactate conversion in breast, but not prostate, cancer cells. NMR Biomed. 2012

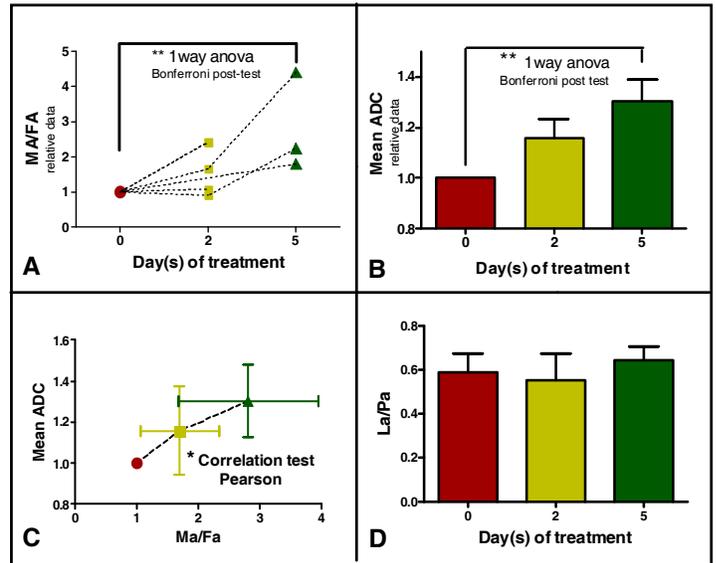


Fig.1 :  $^{13}\text{C}$  flux between Malate (MA) and Fumarate (FA) (A) is increased following Sorafenib treatment. The apparent diffusion coefficient (ADC) is sensitive to Sorafenib (B). Positive correlation between MA/FA and ADC (C).  $^{13}\text{C}$  flux between Lactate (LA) and Pyruvate (PA) (D)

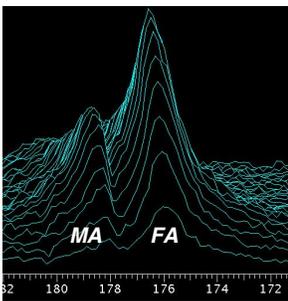


Fig.2 : fumarate (FA) to malate (MA) conversion