In vivo MR detection of inhibition of signaling transduction in non-Hodgkin's lymphoma

S. C. Lee[1], M. Marzec[2], X. Liu[2], S. Wehrli[3], M. Wasik[2], and J. D. Glickson[1]
[1]Department of Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, United States, [3]NMR Core Facility, Children's Hospital of Philadelphia, Philadelphia, PA, United States

INTRODUCTION More and more drugs for cancer are being developed targeting signaling transduction pathways, and some of these agents are already in clinical trials. A noninvasive method for early detection of the effect of these drugs is required. NMR is a promising candidate to meet this need as it can be applied *in vivo* to detect metabolic perturbations in tumors following therapy. We have been investigating effects of the inhibitor of mammalian target of rapamycin (mTOR) which is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, cellular energy demands, and stress. ^{13}C MRS of perfused cells combined with a metabolic network model for quantitating flux could provide an ideal method to measure flux through glycolysis, the pentose shunt and the TCA cycle pathways to identify which pathway is altered by mTOR inhibition. Prior to initiating extensive ^{13}C NMR experiments that are difficult to implement since lymphoma cells are difficult to immobilize in a perfusion chamber, we performed *in vivo* ^{1}H MRS experiments to see if NMR detectable perturbations accompany response to mTOR inhibition. This experiment was based on our previous experience with a human non-Hodgkin's lymphoma xenograft model that demonstrated that lactate concentration in the tumor decreased accompanied tumor cell proliferation following CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy [1] and reports that mTOR inhibition induces tumor growth delay [2].

METHODS We performed *in vitro* and *in vivo* MRS studies. In the *in vitro* study, four different B-cell lymphoma cell lines were investigated; WSU-DLCL2, Val, Ly18 and Ramos. Cultured cells were treated with either the mTOR inhibitor rapamycin or with vehicle under identical conditions. The cells were harvested at 24 h and 48 h and spun down at 4^{o}C. The medium was collected and 12% perchloric acid was poured on to the cell pellets at ice temperature. The mixture was homogenized and centrifuged at 12000 g. The supernatant was neutralized to pH 7.0 with 3M KOH. After removing the precipitate, the sample was lyophilized and dissolved in 0.6 ml D_2O and ^{1}H NMR were obtained with a Bruker 400 MHz high resolution liquid NMR spectrometer. In the *in vivo* study, two different B-cell lymphoma xenografts were examined; WSU-DLCL2, a diffuse large B-cell, and Ramos, a Burkitt lymphoma cell line. The tumor cells were subcutaneously implanted in the upper thighs of immunodeficient mice. When the tumor volumes measured with calipers reached ~500 mm^3, *in vivo* NMR studies were initiated on a Varian 9.4 T /8.9 cm instrument. After acquiring T2-weighted images of the tumor region, two ^{1}H MRS sequences were run; the HDMD-SelMQC sequence [3] for lactate detection and a STEAM sequence for total choline detection. The HDMD-SelMQC sequence imaged lactate slices by placing inversion Hadamard pulses before starting the chemical shift selective SelMQC pulse train which filtered J-coupled lactate signal from below the overlying lipid peaks. For normalization, an HDMD-spin echo sequence of water was employed to measure water in the same slice. The total choline (tCho) signal was detected using STEAM localization. The unsuppressed water signal was acquired from the same voxel for normalization. The MRS measurements were performed before, 48 h and 96 h after initiation of treatments. The rapamycin treatment was 10 mg/kg per dose, 2 doses per day for the first four days and 1 dose per day for subsequent days until day 10 (Ramos) or 14 (WSU-DLCL2). Western blot assays were performed to observe expression of key enzymes.

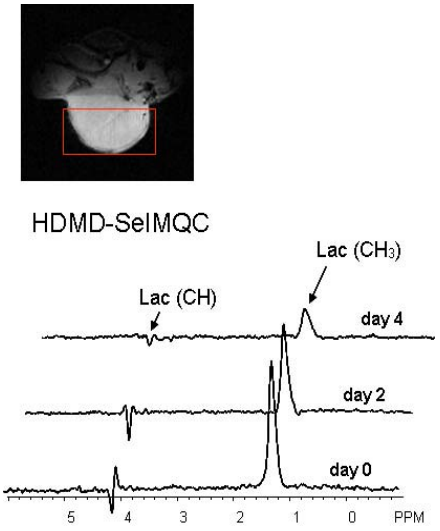

Figure 1. HDMD-SelMQC spectra from a Burkitt lymphoma xenograft at day 0, day 2 and day 4 from initiation of treatment. Rapamycin, an mTOR inhibitor, has been given 10 mg/kg per dose, twice a day.

RESULTS Significant decreases in Lac/H_2O were observed *in vivo* at 48 h and 96 h compared to pretreatment. tCho/H_2O did not change substantially. Tumors exhibited growth retention (WSU-DLCL2) or shrinkage (Ramos), which were observed subsequent to MRS measurements. *In vitro* cell experiment data were in accord with *in vivo* experiments. The activity of Hexokinase 2, a key enzyme in glycolysis, was suppressed indicating suppression of glycolysis by mTOR inhibition.

DISCUSSION The results in this study indicate that inhibition of mTOR signaling transduction can be observed *in vivo* by using ^{1}H NMR to detect lactic acid, the end product of glycolysis. As we have recently translated the multislice SelMQC sequence to a clinical scanner, and showed that tumoral lactate can be measured in cancer patients *in vivo* [4,5], this scheme of detection could eventually lead to tailor-fitting doses of agents that inhibit signal transduction inhibition in individual patients.

REFERENCES 1. Lee et al. In vivo MRS markers of response to CHOP chemotherapy in a human diffuse large B-cell lymphoma xenograft. NMR Biomed 21:723-733 (2008). **2.** Majewski et al., PNAS 97:4285-4290 (2000). **3.** Pickup et al., Lactate imaging with Hadamard-encoded slice-selective multiple quantum coherence chemical-shift imaging. MRM 60:299-305 (2008). **4.** Mellon et al., Detection of lactate with a Hadamard slice selected, selective multiple quantum coherence, chemical shift imaging sequence (HDMD-SelMQC-CSI) on a clinical MRI scanner: application to tumors and muscle ischemia. MRM epub (2009), **5.** Lee et al., Longitudinal study of 1H MRS of lactate upon treatment in a non-Hodgkin's lymphoma patient. ISMRM p.4205 (2009).

ACKNOWLEDGEMENT. NIH 2R01-CA101700.