Monitoring therapeutic response on non-small cell lung cancer in chemotherapy by amide proton transfer (APT) imaging in mice

Keisuke Ishimatsu[1], Shanrong Zhang[1], Koji Sagiyama[1], Osamu Togao[1], Brenda Timmons[2], John Minna[2], and Masaya Takahashi[1]

[1]*Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States,* [2]*Hamon Center for Therapeutic Oncology, Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, United States*

Target audience: Researchers and clinicians interested in molecular imaging of cancer.

Introduction: The development of new and effective therapies for patients with advanced lung cancer remains a major health imperative, which requires reliable evaluation methods (1). We previously demonstrated that the amide proton transfer (APT) imaging could distinguish the different types of lung tumors where the APT signal was higher in a tumor which exhibited more active proliferation than the other (2) and that the APT signal increased in the normal lung cells when oncogene was introduced (3). It has been demonstrated in the animal models that the APT imaging provided a useful imaging biomarker on the brain tumors for monitoring the therapeutic effects of radiotherapy (4) and chemotherapy, which predicted the treatment outcomes prior to the anatomical changes (5). The objective of present study was to investigate if APT signal is useful for evaluating treatment responses of the lung tumors in chemotherapy.

Materials and Methods: *Animal Protocol:* Genetic alterations in the kinase domain of the epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC) patients are associated with sensitivity to treatment with small molecule tyrosine kinase inhibitors (1). Nine mice were subcutaneously implanted $1x10^6$ NSCLC cells (HCC827) into the thigh. When the tumor was grew up to the volume of ~500 mm^3 at 5 weeks after implantation, all animals were subjected to the first MRI session (Day0) and divided into two groups as follows: either 12.5 mg/kg erlotinib which is a first-generation small molecule EGFR tyrosine kinase inhibitor (EGFR-TKI: N=5) or vehicle (phosphate buffered saline: PBS, N=4) was orally administrated daily. The same MRI session was repeated every 7 days thereafter. The tumors were harvested after the final MRI session for histology.

MRI: All MRI sessions were conducted in a 7-T small animal MR system (Varian, Inc, Palo Alto, CA) with a 40 mm (I.D.) radiofrequency (RF) coil. Animals were anesthetized with 1%-2% isoflurane (AERRANE, Baxter Healthcare Corporation, IL) mixed in 100% oxygen. First, low-resolution localizer imaging was performed to confirm reproducible positioning. High-resolution axial multislice T2-weighted images (T2WI) were obtained on entire tumor using a fast spin-echo sequence (TR/TE = 2500/60 msec; FOV = = 30×30 mm; matrix size = 128×128; slice thickness = 1 mm; gapless; NEX = 4). On a single 1-mm-slice delineating the maximum diameter of the tumor, APT imaging was performed as follows: Gradient echo images were obtained following a presaturation pulse (continuous-wave: CW block pulse, B_1 = 2.3 μT, duration = 5 s) which was applied at 29 frequency offsets from 7 to -7 ppm with an interval of 0.5 ppm. Other imaging parameters were: TR/TE = 5.32/2.64 ms, flip angle = 20°, FOV = 30×30 mm, matrix = 128 × 64 (reconstructed to 128×128), NEX = 8. A control image was obtained with the presaturation pulse at 300 pm. Water saturation shift referencing (WASSR) images were collected for B_0 inhomogeneity correction with a CW pulse (B_1 = 0.2 μT, duration = 200 ms) which was applied at 31 frequency offsets from 1.5 to -1.5 ppm every 0.1 ppm. Total acquisition time for each animal was approximately 40 min.

Image Analysis: Tumor volumes were measured on the T2WI. The z-spectra were fitted through all offsets on a pixel-by-pixel basis according to the procedure using the 12th-order polynomial fitting followed by the correction for B_0 inhomogeneity as previously reported (6). MTR asymmetry (MTR_{asym}) was defined as: $MTR_{asym} = S_{sat}(-offset)/S_0 - S_{sat}(+offset)/S_0$, where S_{sat} and S_0 are signal intensities on the images with presaturation pulse at 7 to -7 ppm and control (300 ppm), respectively. The calculated MTR_{asym} map at the offset of 3.5 ppm is called the APT-weighted image (APTWI). Region-of-interests (ROIs) were carefully placed around the edge of the tumors on APTWI to measure APTR. APTR was also measured in normal appearing muscle for a reference in each mouse. Corrected APTR was calculated as the difference between these two APTRs (tumor – muscle).

Results and Discussion: There was no significant difference in volume (mm^3) between the groups (erlotinib: 473.1±148.7; control: 614.5±276.5) at Day0. The tumor volume substantially reduced with 1 week of treatment and stayed constant in the erlotinib group (Fig. 1). All five animals survived in general health condition through the protocol. By contrast, the tumor volume increased rapidly in the control group (Fig. 1) and thus all animals should have been sacrificed on Day14 due to the ethical criteria. Figure 2 shows the typical APTWIs in the both group at Day0 and Day7. The APT signal reduced along with reduction of the tumor volume in the erlotinib group whereas it increased inhomogeneously with tumor growth in the control group. Figure 3 demonstrates temporal change (%change) in corrected APTR relative to that at Day0 in both groups. The corrected APTR in all animals decreased by the erlotinib treatment and stayed constant while it increased substantially in the control group. The present study indicated that APTR could be a useful biomarker for monitoring treatment responses of the lung tumor in chemotherapy, which was consistent with previous results in the brain tumor (5). Further the result might imply that the early APT response could also be a prognostic imaging biomarker. As APT imaging is highly responsive to treatment effects and has prognostic potential, further studies to evaluate APT response in many combinations of lung tumor cell lines and different types of treatment are significant. Moreover, by establishing such methods in the animal models with knowledge of genomic aberrations, we may be able to screen potential drugs to prevent progression and/or promote regression of lung cancer.

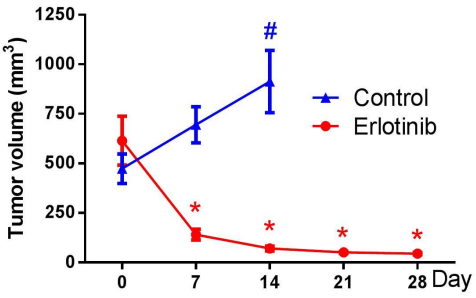

Fig 1. Temporal change in tumor volume in the control and erlotinib groups. The tumor volume increased in the control group while it decreased in the erlotinib group. * and #, P < 0.05 vs. baseline (Day0) by Dunnett's multiple comparison test.

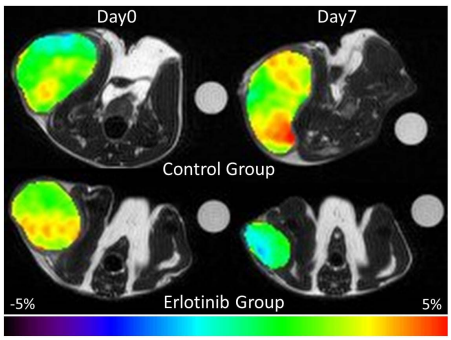

Fig 2. Typical APT-weighted images of the control (top) and the erlotinib (bottom) groups at Day0 and Day7. The APT signal of tumors increased in the control group while it decreased after treatment in the erlotinib group.

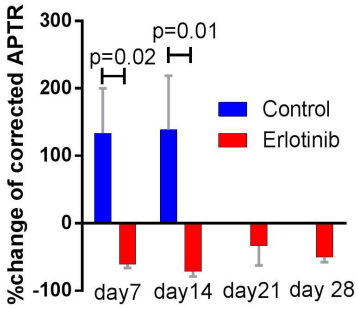

Fig 3. Temporal change (%change) in corrected APTR (MTR_{asym} at 3.5 ppm) relative to that at Day0. The APTR rapidly reduced and stayed constant after treatment in the erlotinib groups. P value, by Student's t-test.

References: 1. Li D et al. Oncogene 27: 4702 (2008). 2. Togao O et al. PLoS One. 8:e77019 (2013). 3. Kim K et al. #4373 Proc. ISMRM (2012). 4. Zhou J et al. Nat Med 17:130 (2011). 5. Sagiyama K et al. Proc Natl Acad Sci 111:4542 (2014). 6. Salhotra A et al. NMR Biomed 21:489 (2008)