Assessment of spin echo BOLD fMR imaging as a prognostic tool for determining chemotherapeutic efficacy.

A. Bhattacharya[1], R. Mazurchuk[2], J. A. Spernyak[3], S. Cao[4], F. A. Durrani[4], Y. M. Rustum[5]

[1]Preclinical Imaging Resources, Roswell Park Cancer Institute, Buffalo, NY, United States, [2]Preclinical Imaging Resources, Roswell Park Cancer Insitute, Buffalo, New York, United States, [3]Preclinical Imaging Resources, Roswell Park Cancer Institute, Buffalo, New York, United States, [4]Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, New York, United States, [5]Cancer Biology, Roswell Park Cancer Institute, Buffalo, New York, United States

Introduction – A non-invasive, surrogate marker of response to cancer chemotherapy can lead to improvement in interventional options at early stages of chemotherapy. A MR imaging method that can detect chemotherapy-induced changes in tumor vasculature, stromal bed and increased cell kill would be a valuable tool for predicting chemotherapeutic response that can impact cancer patient care considerably. These studies were done to determine feasibility of using T_2 -weighted spin echo (SE) based BOLD fMR imaging using RARE phase encoding for predicting chemotherapeutic response in mice bearing HNSCC xenografts.

Methods – Nude mice bearing HNSCC xenografts - A253 (less vascularized, up to 60% complete response) and FaDu (more vascularized, 100% complete response) were treated with 5-methylselenocysteine (MSC) daily @ 0.2 mg/mice/day per oral for a total of 35 days starting 7 days prior to CPT-11 therapy (100 mg/kg/week x 4 intravenous). Mice were anesthetized using intraperitoneal 100 mg/kg ketamine HCl/10 mg/kg xylazine and imaged with T_2 -weighted SE RARE imaging sequence (TE = 79.7 ms, TR = 4622.5 ms, NEX 2, FOV 3x3 cm, 1 mm thick axial slices perpendicular to the spinal axis, with an in-plane spatial resolution of 234 μm). Sequential BOLD fMR images were acquired of the mice breathing first room air (4.5 minutes) followed by carbogen (93% O_2 + 7% CO_2) for 9 minutes using a 4.7T imaging spectrometer. Percent change from baseline was calculated on a pixel-by-pixel basis for regions visually identified as containing tumor. fMR image intensity changes from pre and post carbogen paired T_2 weighted MR images were calculated using the equation: % Signal Intensity Change = {(post – pre)/(pre)} x 100, where 'pre' refers to the T_2 weighted images obtained with the mice breathing room air, and 'post' refers to images acquired while breathing carbogen. Serial scans during treatment were done on days [(-7), pre-MSC], [(0), pre-CPT-11], 5, 12, 19 and 26. The percentage of voxels with positive fMR signal change was determined for each animal and timepoint, and changes in fMR voxel populations were analyzed with linear regression and one way ANOVA. The mean fMR signal change was also analyzed for significance using Mann Whitney two-tailed unpaired t-test. GraphPad InStat (Version 3.01 for Windows 95, GraphPad Software, San Diego, Ca) was used to do all analysis and a p value $\leq$ 0.05 was considered statistically significant.

Figure 1. Linear regression analysis between CRs and non-CRs

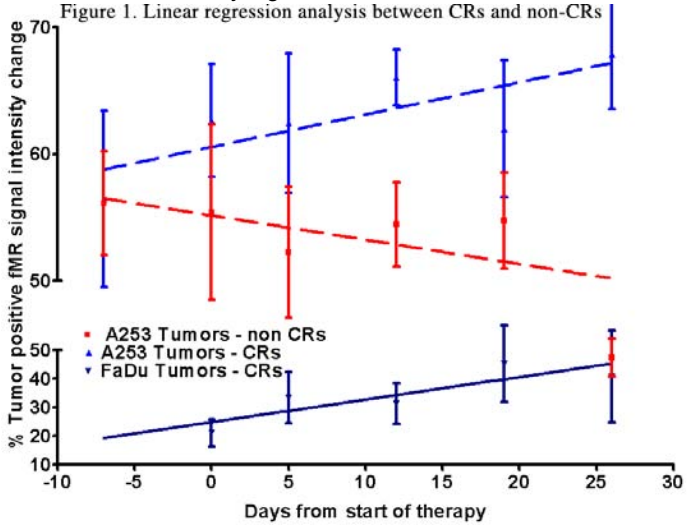
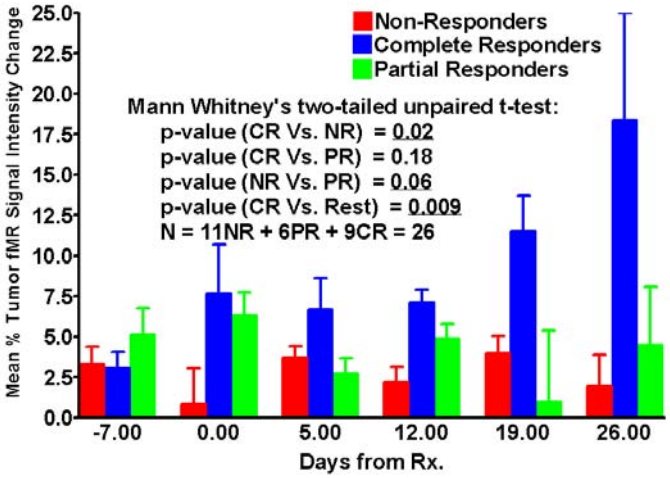


Figure 2. Response to MSC + CPT-11 therapy in A253 - CRs *vs.* NRs *vs.* PRs



Results – All FaDu tumors and 35% of A253 tumors had a complete remission. Linear regression analysis for FaDu and complete responders (CRs) in A253 (Figure 1) followed similar trends of increasing percentage of voxels with positive fMR signal, which was a statistically significant difference when compared to non-CRs in A253 (P_{slope} = 0.088 and $P_{Elevation}$ = 0.001). Mean tumor percent fMR signal intensity change (Figure 2) was significantly higher in CRs vs. non-CR's on day 19 and 26 ($p < 0.05$). Further analyses between the three A253 tumor groups using one-way ANOVA revealed day 12 (P = 0.02) and day 26 (P = 0.03) as prognostically significant time points predicting for therapy response. Furthermore, the percentage of voxels that had an fMR signal intensity change 2 standard deviations below the mean was significantly (p = 0.09) reduced in A253 CRs *vs.* non-CRs on day 5, with FaDu tumors showing a similar trend as well. The reduction in the volume of tumor with negative fMR signal change is suggestive of lack of neoangiogenesis[1]. On day5, tumor regression as measured with calipers was only 20% and 13% for CR and PR respectively while the NR group showed an increase in tumor weight by 9%.

Conclusions - Despite variances in the intratumoral morphology in the tumor types, spin-echo based BOLD fMR imaging was successful in demarcating the CRs from the non-responders. The increase in mean fMR signal intensity change in CRs during therapy reflects the intratumoral milieu of terminally well-differentiated cells and wound healing response in a tumor undergoing remission. Decrease in percent fMR signal intensity change in tumor has been shown by us earlier to be correlated to tumor neoangiogenic microvessel density using the same fMR scan protocol[1]. The increase in fMR signal intensity in responders may thus reflect the reduction of existing and non-recruitment of new neoangiogenic vessels by the responding tumors. Earlier, we had shown that MSC has antiangiogenic properties[2] that may play a critical role in tumor regression. In contrast, the non-responders appear to have fresh recruitment of neoangiogenic blood vessels, which is reflected in their successful survival against this treatment protocol. Using this method we were able to show a significantly varying trend in the CRs as early as on day 5 of therapy, indicating prognostic potential.

References – 1) Bhattacharya *et al*, Lack of microvessels in well-differentiated regions of human head and neck squamous cell carcinoma A253 is associated with functional magnetic resonance imaging detectable hypoxia, limited drug delivery and resistance to irinotecan therapy. Clin Can Res, 2004,10:8005-8017. 2) Yin *et al*. Potential involvement of suppressed cycloxygenase-2, inducible nitric oxide synthase and hypoxia-inducible factor 1α expression in modulation of irinotecan sensitivity by Se-methylselenocysteine. Oncogene, 2005, in-press.