Multimodal Imaging of a Dual PI3K/mTOR Inhibitor Demonstrates Strong Effects on Vascular Function

S. K. Wyatt[1], K. H. Barck[1], J. R. Oeh[2], H. Bou-Reslan[1], T. C. Cao[1], H. Koeppen[3], L. S. Friedman[2], D. Sampath[2], and R. A. Carano[1]
[1]Biomedical Imaging, Genentech, Inc, South San Francisco, CA, United States, [2]Translational Oncology, Genentech, Inc, South San Francisco, CA, United States, [3]Pathology, Genentech, Inc, South San Francisco, CA, United States

Objectives. The PI3K/mTOR pathway represents a well-established oncology target that has both direct tumor and vascular effects. It has been shown to mediate both vascular endothelial growth factor (VEGF)-driven endothelial cell survival and vascular permeability [1]. Previously, Schnell *et al* reported a reduction in the volume transfer constant K^{trans} assessed by dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) following treatment with a dual PI3K/mTOR inhibitor (PI3K/mTORi) [2]. Since K^{trans} is sensitive to both blood flow and permeability, the effects on blood flow remain unknown. Thus, the aims of this study were threefold: (1) confirm that dual PI3K/mTOR inhibition results in K^{trans} suppression, (2) elucidate the role of PI3K/mTOR signaling on the DCE-MRI fractional plasma volume parameter (v_p), and (3) determine whether PI3K/mTORi specifically effects blood flow using a DCE-ultrasound (DCE-US) contrast agent that remains intravascular.

Methods. *Animal Model:* Animal procedures were approved by the institutional AAALAC-accredited review board. Two cohorts of athymic nude mice were inoculated subcutaneously on the hind limb with $3.5x10^6$ HM7 (human colorectal cancer) cells for independent DCE-MRI and DCE-US studies. *Multispectral (MS) DCE-MRI.* MRI was performed on a Varian 9.4T MRI system with a Varian volume transmit (63mm)/surface receive (20mm) coil setup. Twelve coronal, 1mm-thick slices were acquired with a 25.6x25.6mm field of view and 64x64 pixel resolution for ADC and T_2 mapping. A multi-slice, diffusion-weighted fast spin-echo imaging sequence was used to obtain ADC measurements (6 b-values from 82-1129s/mm^2), TR=3.7s, ETL=4, NEX=2, δ=3.3ms, Δ=30ms). T_2 and M_0 maps were acquired using a multi-slice, spin-echo imaging sequence (TE=5,26,47,68 ms, TR=3s and NEX=1). Pre-contrast 3D gradient echo (3DGE) datasets were acquired at 2° and 10° flip angles, TR=6ms, TE=1.26ms, NEX=4, FOV=25.6x25.6x16mm, matrix=64x64x16. A bolus injection of Omniscan (1mmol/kg in 100µL) was injected via a tail vein catheter following collection of the pre-contrast images. Post-contrast 3DGE images were then acquired approximately every 9s for 30min (10°, TR=6ms, TE=1.26ms, NEX=1). The DCE-MRI analysis of K^{trans}, v_p and v_e was restricted to the viable tumor tissue using a multispectral approach [3,4]. *DCE-US:* An Acuson Sequoia C512 system (Siemens Medical Solutions) and a 15L8-S probe was used for harmonic US imaging with the following parameters: P14 MHz, -10dB, MI 0.21, axial and lateral resolution of 34µm and a frame rate of 20 frames/second. A vascular microbubble contrast agent (DEFINITY, Lantheus Medical Imaging) was delivered via a jugular vein catheter at a constant 3µL/min infusion rate using a syringe pump. Following bubble destruction from a high-intensity pulse, the reflow kinetics were estimated and fit to a kinetic model [5]. Enhancement factor was defined as the percentage of tumor area above a minimum blood flow threshold. *Experimental details:* DCE-MRI was performed pre- and 24h post-treatment with 10mg/kg GDC-0980 (n=9) or methylcellulose/Tween-80 (MCT vehicle control, n=10). DCE-US was performed in a second cohort of mice 24h post-treatment with 10mg/kg GDC-0980 (n=6) or MCT (n=6).

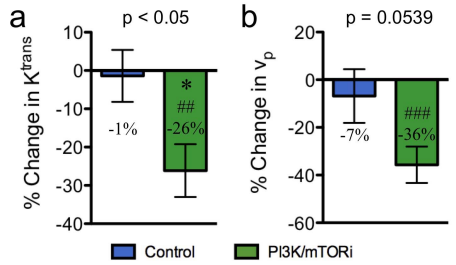

Fig 1. % change in (a) K^{trans} and (b) v_p 24h post-tx with MCT or PI3K/mTORi (avg± SEM). * p<0.05 t-test vs control. ## p<0.01, ### p<0.005 paired t-test vs pre-tx.

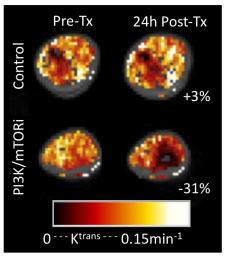

Fig 2. Representative viable tumor K^{trans} maps overlaid onto their corresponding M_0 images pre-tx and 24h post-tx with MCT or PI3K/mTORi.

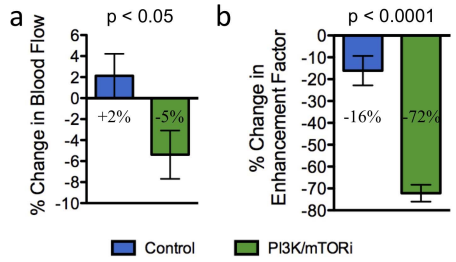

Fig 3. % change in (a) blood flow or (b) enhancement factor 24h post-tx with MCT or PI3K/mTORi (avg± SEM).

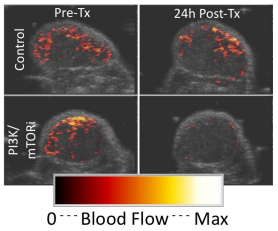

Fig 4. Representative blood flow maps overlaid onto their anatomical images pre-tx and 24h post-tx with MCT or PI3K/mTORi.

Results and Discussion. Overall, MS DCE-MRI demonstrated significant tumor growth suppression as well as confirmed the reduction in K^{trans} and suggests the potential for v_p changes in response to dual inhibition of PI3K/mTOR (Fig 1,2). More specifically, vehicle-treated MS viable tumor tissue grew an average of 46±18mm^3 in 24h, while PI3K/mTORi-treated viable tumor volume remained static (-5±9mm^3; p<0.0001). PI3K/mTORi-treated tumors demonstrated a significant 26% reduction in K^{trans} relative to pre-treatment (p<0.01), which was significantly lower than the vehicle-treated tumors at 24h (-26% vs -1%, p<0.05; Fig 1a,2). In addition, a single dose of a dual PI3K/mTORi resulted in a significant reduction of v_p relative to baseline values (-36%, p<0.005) and a strong trend toward a reduction in v_p relative to the changes observed in the vehicle-treated tumors (p=0.0539; Fig 1b). The DCE-US study demonstrated a decrease in blood flow (PI3K/mTORi: -5±6%, Control: 2±5%, p<0.05; Fig 3a,4) within the enhancing tumor, which is consistent with VEGF-dependent vasoconstriction. Furthermore, PI3K/mTOR inhibition resulted in a 72% reduction in the DCE-US enhancement factor parameter (p<0.0001; Fig 3b). The reduction in enhancement factor as well as a strong trend toward a reduction in v_p may be attributed to a reduction in vessel density and loss of small vessels through PI3K/mTORi as seen in an independent vessel size imaging study [6]. Combined, these results help elucidate the effects of PI3K/mTOR inhibition on tumor vasculature and advocate the use of these imaging techniques as biomarkers for these therapies.

[1] Fukumura *et al*., PNAS 2001: 2604-2609. [2] Schnell *et al*., Cancer Res. 2008: 6598-6607. [3] Carano *et al*., MRM. 2004: 542-551. [4] Berry *et al*., MRM. 2008: 64-72. [5] Wei *et al*, Circulation. 1998: 473-483 [6] Wyatt *et al*., ISMRM 2011, submitted.