Magnetic Resonance Imaging at 7 T for Correlation of Therapy-Induced Alterations in T_2 intensity, ADC and Tumor Volume in Ewing's Sarcoma Xenografts

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Introduction: Sarcomas account for 10% of pediatric diagnoses, 8% of cancers in the young population and 1% of adult cancers (1). This diverse group of malignancies is often lethal in surgically unresectable, recurrent or metastatic settings. Chemotherapy has at times demonstrated clinical benefit for advanced disease; however, the prognosis for soft tissue sarcoma patients remains poor with a disease-free survival at 5 years less than 10% underscoring the need for novel therapeutic treatments. Previously, we explored combinations of cytotoxic and targeted agents in rhabdomyo-, osteo- and Ewing's sarcoma by cell viability and apoptosis assays. Based on the findings, the therapeutic effect of Dasatinib (multitarget kinase inhibitor) and Triciribine (AKT inhibitor) were evaluated in Ewing's Sarcoma mouse xenografts by magnetic resonance imaging (MRI) at 7T.

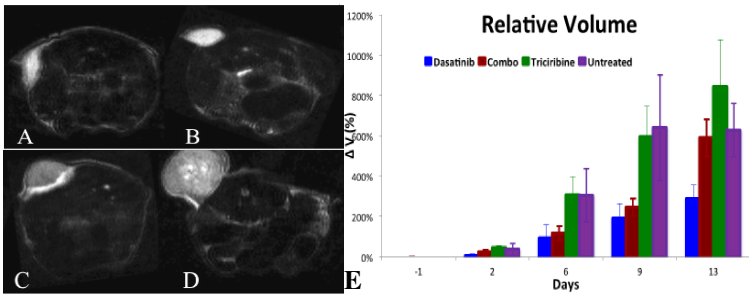

Fig 1. T_2-weighted FSE images (R=312 µm) showing Das (A), Comb (B), Tri (C) and Ctrl (D) at day 6 and corresponding tumor volumes for all animals (E).

Methods: 24 mouse xenografts with A673 Ewing's Sarcoma were divided into a control group, two groups receiving either Dasatinib (Das) or Triciribine (Tri), and a combination of both drugs (Comb). Treatments were administered daily with 200 mg/kg Das in a citrate solution orally, and/or Tri at 2 mg/kg by IP injection in a 40% DMSO solution with PBS equaling 100ul. Evaluating tumor volume, apparent diffusion coefficients (ADC) and T_2 signal intensities, MR imaging was performed on days -1, 2, 6, 9 and 13. Prior to imaging, mice were anesthetized with 1% isoflurane in O_2 and placed into an insertion cradle fitted with a respiratory pad beneath the animal. Body temperature was monitored with a fiber-optic rectal probe and maintained at 37 °C while being controlled using a small animal monitoring system (SA Instruments, NY). All imaging was performed using a 7 T horizontal bore ASR 310 MRI instrument (Agilent Technologies, CA) equipped with actively shielded gradients capable of 400 mT/m gradient strength. Using a 35 mm-inner-diameter Litzcage coil (Doty Scientific, Inc), axial T_2-weighted fast spin-echo (FSE) images were obtained with TR/TE = 2400/72 ms, field of view of 40x40 mm, matrix size of 128x128 and 15 slices at 1.25 mm. Similarly, diffusion-weighted datasets were acquired with TR/TE = 1800/36 ms and b=[50, 500, 1000, 2000, and 6500]. The data were analyzed using in-house scripts coded in Matlab (Mathworks, Inc., MA). Following MRI, animals were euthanized and tissue prepared for histological staining with hematoxylin and eosin (H&E).

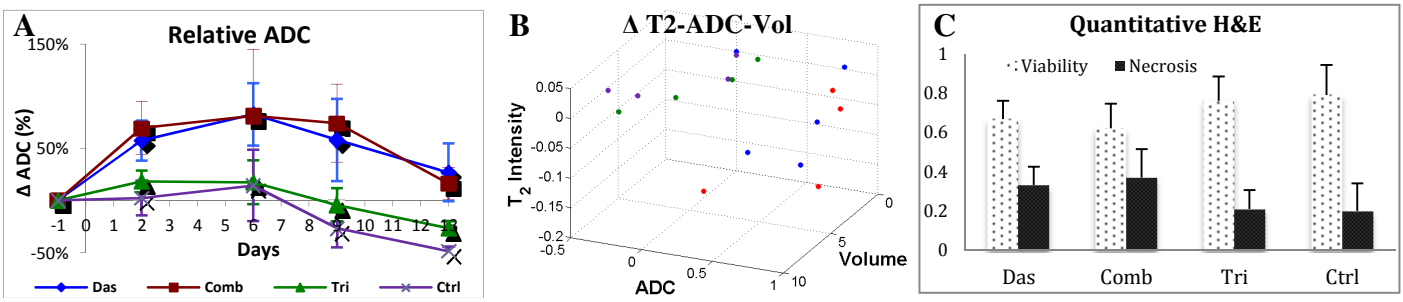

Fig 2. A) percent change in ADC compared to baseline, B) correlation between volume, ADC and T2 intensity and C) quantitative H&E histology.

Results & Discussion: Demonstrated in Fig 1E, similar tumor growth was observed for all animals initially. On day 6, the Ctrl and Tri groups showed notable increases in growth compared to Das and Comb. In fact, following the fourth treatment on day 2, Das demonstrated significantly smaller tumor volumes than Tri at all time points. Similarly, the Comb also showed significantly lower volumes following the treatment on day 6 in comparison to Tri and Ctrl. In corroboration, the ADC values of Das and Comb demonstrated significant increases immediately following treatment while the Ctrl and Tri showed similar values throughout the experiment (Fig 2A). While increases in ADC may result from drug therapy, the distribution of ADCs (*i.e.* skewness and kurtosis) within the tumor also should be expected to shift. Shortly following the first treatment, skewness and kurtosis for the two responsive groups (*i.e.* Das and Comb) altered substantially compared to the Ctrl and Tri. Statistically significant differences were maximal at day 6. Interestingly, correlation of changes in T_2 intensity, ADC and tumor volume demonstrated an obvious pattern where Das and Comb again were grouped together (Fig 2B). While the Tri and Ctrl showed no obvious changes in T_2 intensity with treatment and growth, Das and Comb show an obvious correlation between these characteristics suggesting that alterations in T_2 in addition to ADC may serve as an early indicator of treatment response. Quantitative histological analysis demonstrated an overall higher amount of necrosis in the Das and Comb groups than in the two other groups. Meanwhile, the Ctrl and the Tri displayed an overall lower amount of necrosis, which reached statistical significance as compared to Das and the Comb. In addition, significantly higher cell viability was observed in the Ctrl as opposed to the Comb group.

References: 1. Reed D *et al*, (2011), *Cancer control : JMCC.* 18(3):188-195.