MRI at 7 T Correlates Therapy-Induced Alterations in T2 heterogeneity, ADC and Tumor Volume in Ewing's Sarcoma Xenografts

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Introduction: Ewing's sarcoma (ES) is one of the most aggressive human malignancies and accounts for 8% of primary malignant bone tumors[1]. ES mainly affects children 10-20 years of age and while progress has been made in the past decades, the prognosis remains poor underscoring the need for novel therapeutic treatments. Importantly, a non-invasive imaging approach that can accurately assess therapy-induced response in ES is yet to be established. Previously, we explored targeted agents in rhabdomyo-, osteo- and Ewing's sarcoma and found that Dasatinib (DAS) combined with triciribine (TCN) demonstrated significant synergy across cell lines, as well as in ES mouse xenografts[2]. The current study further evaluates therapeutic effects of DAS and TCN in ES xenografts using MRI at 7 T and a profound analytical approach for assessing response.

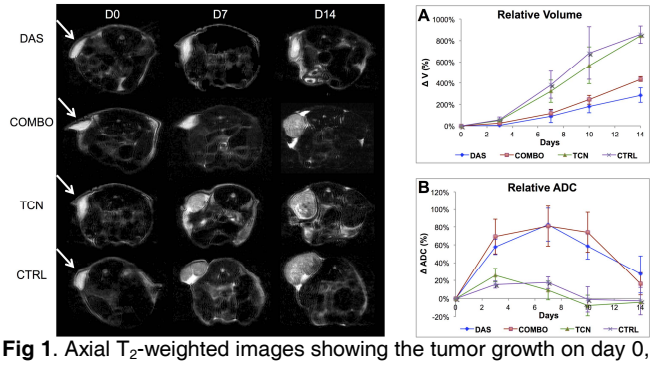

Fig 1. Axial T_2-weighted images showing the tumor growth on day 0, 7 and 14. **(A)** Percent change in average tumor volume compared to day 0 and **(B)** corresponding change in ADC values.

Methods: 24 male mice (Balb C–Nu/Nude) received subcutaneous flank injections of $1x10^6$ A673 sarcoma cells transfected with luciferase, 50 □L PBS and 50 □L Matrigel. Tumor growth was monitored with MRI and treatments initiated at a volume of 80 mm^3. Prior to drug administration, xenografts were divided into four groups; controls (Ctrl), Dasatinib (DAS), Triciribine (TCN) and DAS+TCN (Combo). Treatments were administered daily with 200 mg/kg DAS in a citrate solution orally, and/or TCN at 2 mg/kg by IP injection in a 40% DMSO solution with PBS equaling 100 µl. MRI was performed on day 0, 3, 7, 10 and 14 using a 7 T horizontal bore ASR 310 scanner (Agilent Technologies Inc., CA) with actively shielded gradients (400 mT/m). Mice were anesthetized with 1% isoflurane in O_2 and placed into an insertion cradle. Temperature and respiration were monitored using an animal monitoring system (SA Instruments, NY). 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]. Image reconstruction and volumetric analysis was performed in VnmrJ (Agilent Technologies) while apparent diffusion coefficients (ADC), area under the curve (ADC-AUC) and T_2 edges were calculated in MATLAB.

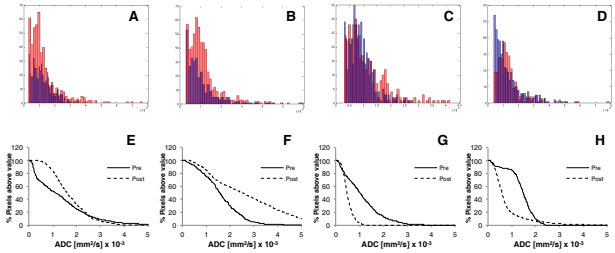

Fig 2. (A-D) Histograms demonstrating ADC distribution pre- (blue) and post-treatment on day 3 (red) for select animals. (E-H) Corresponding incremental pixel fraction plots showing a shift of ADCs towards higher values in Das & Combo than TCN & CTRL.

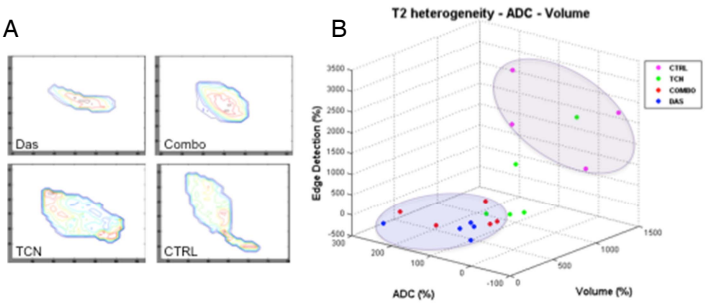

Fig 3. (A) Contour plots obtained from T_2-weighted data show major edges within the tumors, indicating a higher degree of heterogeneity in CTRL and TCN than in DAS and COMBO. **(B)** 3D graph of T_2 edge detection (i.e. heterogeneity), mean ADC and tumor volumes showing the spatial clustering of the CTRL versus the responsive groups (DAS and COMBO).

Results & Discussion: T_2-weighted datasets indicated that DAS and Combo experienced growth inhibition compared to TCN and CTRLS (Fig 1). Quantitative analysis confirmed that DAS and DAS+TCN significantly inhibited tumor growth compared to TCN and controls and that these changes were statistically significant by day 10 (Fig 1A). Importantly, significant increases in ADC were observed for DAS and Combo by day 3 (Fig 1B). Providing information that may be masked by mean ADC, ADC skewness and kurtosis pre- and post treatment showed significant shifts in both properties for DAS and Combo while no notable changes for TCN and CTRL were detected (Fig. 2 A-D). Introducing a sensitive approach for assessing altered ADC, pixel fraction plots were generated for pre- and post treatment and compared across animals (Figures 2 E-H). In agreement with the mean ADC, these graphs demonstrated shift of ADCs towards higher values for Das and Combo while TCN and CTRLS decreased. To capture the heterogeneous tumor microstructure that was particularly noticeable at larger volumes, edge detection analysis was performed and indicated larger variation in TCN and CTRL than in DAS and Combo. In agreement with previous findings that correlate tumor heterogeneity following therapy with a poorer outcome[3], quantitative analysis of these demonstrated statistical significance by day 3. Interestingly, and subject for further analysis, our 3D analysis combining the change in T_2-based heterogeneity, tumor volume and mean ADC post-treatment yielded distinct clusters separating controls from the responsive treatments (DAS and Combo).

References: **[1].** Ross KA. et al., ISRN Oncol. 2013;2013:759725. **[2].** Cubitt CL., et al, Sarcoma, 2013. **[3].** Ahmed A. et al, MRI, 2013.