Evaluation of diffusion MR as a biomarker for nanoparticle therapy response in lymphoma

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Objective
To evaluate the applicability of diffusion MR as a viable biomarker of early treatment effects of the targeted nanoparticle therapy IT-101.

Introduction
Relapsed or refractory lymphoma remains an impediment for long term disease free survival, with over 50% of patients with aggressive non-Hodgkin lymphoma and advanced indolent lymphoma requiring salvage therapies [1, 2]. However, not all patients respond well to salvage therapies that are currently adopted, highlighting a need for other strategies. IT-101, a nanoparticulate conjugate of the topisomerase inhibitor 20(S)-Camptothecin (CPT), has recently been shown to be effective in solid tumors [3] and a variety of lymphomas [1]. As this therapy moves into clinical trials, it would be desirable to have a non-invasive biomarker that could monitor the early response of the tumor mass to therapy prior to macroscopic size changes. Diffusion MR has shown promise in monitoring treatment efficacy for conventional chemotherapy treatment in a variety of tumor models [4, 5]. Here, we evaluate whether this imaging biomarker is sensitive to IT-101 treatment in a mouse model of lymphoma.

Methods
Animal: Athymic nude mice were injected with $5x10^6$ Daudi (human Burkitt lymphoma) cells in a 0.2mL of 1:1 mixture of tumor cell suspension in 1% human serum albumin in HBSS (Mediatech) and Matrigel (BD Biosciences) into the thigh. A subset of the animals had tumors injected bilaterally. Studies started ~3 weeks post injection, when tumor sizes reached ~4-800mm^3. Imaging: Imaging was done on a 7T Bruker Biospec MR system. For each imaging session, an anatomical image (RARE TR/TE = 4000/22ms) and a diffusion MR image (Spin-echo TR = 3-3500ms, [depending on tumor size], TE = 24ms, b = 0, 800, 1200 s/mm^2) were obtained. Experimental: A baseline image was obtained on Day 1. After imaging, 5mg/kg of IT-101 was injected via a tail vein for treated animals (N = 6). Volume matched saline was i.v. injected for controls (N = 4). The cohorts were divided evenly between single and bilateral tumor bearing mice. Imaging was repeated 3 and 5 days post injection. Apparent diffusion coefficient (ADC) maps were generated with in-house developed software using MATLAB. ADC tumor analysis was done using manually drawn ROIs defined by the generated S_0 images.

Results
Tumor histograms comparing ADC changes over the course of a week between treated and control are shown in Figure 1. A discernable right shift of the histogram towards higher ADC values can be seen over the week for the treated mouse whereas the control ADC histograms did not. A three way ANOVA with repeated measures analysis of the mean ADC values percentage change (normalized to baseline) show a significant difference between the control and treated cohorts ($p < 0.02$) but not between bilateral and single tumors ($p = 0.7$), with a corresponding noticeable decrease in size ($p = 0.06$). When compared separately to baseline ADC values, the treated tumors show a significant increase in ADC on both day 3 ($p < 0.01$) and day 5 post treatment ($p < 0.01$). In contrast, ADC values for control tumors were not significantly different on day 3 ($p > 0.1$) and or day 5 ($p = 0.1$), as shown in Figure 2. During the same period, image derived size measurements show a non-significant change in size for both cohorts on day 3 ($p > 0.4$), but a significant decrease (-25±7%, $p < 0.05$) for the IT-101 tumors and increase for control tumors (26±18%, $p = 0.2$) on day 5. An ADC map series of a treated tumor is shown in Figure 3, showing a diffuse pattern of ADC increase within the tumor.

Discussion
IT-101 treated lymphomas consistently show significantly increased mean ADC values compared to baseline and controls. This can be seen as early as day 3 of treatment, before a significant size change is discerned. The enhanced activity of IT-101 is due to its preferential accumulation in the tumor via the EPR effect [6]. Here, the lack of a significant difference of ADC changes between single and bilateral tumors suggests that: a) Even at a low dose, the plasma concentration of the drug remains significant and shows enhanced tumor targeting; b) The tumor microenvironment modulates the uptake of this nanoparticle therapy, as reflected in the ADC maps. Studies are ongoing to ascertain this relationship of particle dose uptake and response via *ex-vivo* and *in-vivo* assays.

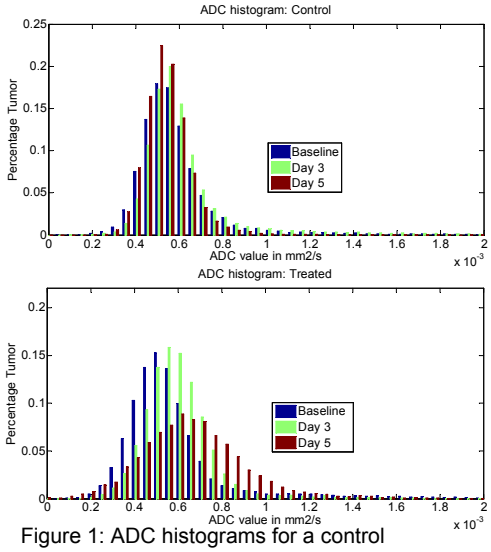

Figure 1: ADC histograms for a control mouse (top) and IT-101 treated mouse (bottom). Note the increase trend of the IT-101 ADC histogram during treatment course.

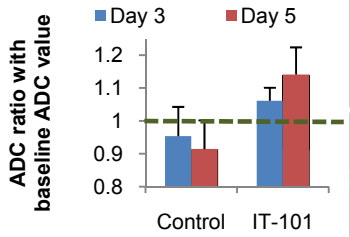

Figure 2: ADC changes from baseline. IT-101 treated animals show increases in ADC values on both day 3 and 5 post treatment ($p < 0.02$). Controls showed ADC decrease on both days ($p = 0.1$). Green line denotes unity.

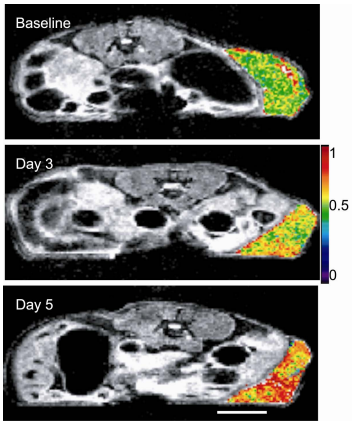

Figure 3: ADC maps for a treated animal. A steady increase in the ADC values can be seen over the 5 day treatment course. Distribution of the increase is heterogeneous, suggesting the modulation of IT-101 uptake by the tumor environment (Scale bar = 5mm, color bar → $x10^{-3}$ mm^2/s)

References
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