**Ferumoxytol-enhanced MRI of macrophages in CHL-1melanoma tumor model**

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**Introduction:** Previously, ferumoxytol-enhanced MRI was used to detect macrophages in a mammary carcinoma xenograft tumor model (1). In this study, we further investigated the optimal ferumoxytol concentration, MRI detection scheme and timeframe of macrophages in CHL-1 melanoma xenograft tumor bearing mice.

**Method:** All procedures were approved by the Animal Care and Use Committee of GlaxoSmithKline and were specifically designed to minimize animal discomfort. 8 weeks old C.B-igh-1/IcrTac-Prkdc<scid> nude female mice (n=12) bearing CHL1 (melanoma) tumors were used in the study. In this strain of mice, T and B cells are deficient. The study was conducted in 2 weeks: during the 1st week mice were imaged pre-contrast (W1-t0) and at 1hour (W1-t1) and 24 hours (W1-24h) post iv injection of 0.5 mmol [Fe]/kg ferumoxytol; during the 2nd week; mice were imaged pre-contrast (W2-t0) and at 24 hours (W2-24h) post iv injection of 1 mmol [Fe]/kg ferumoxytol. All experiments were performed on a 9.4T/30 cm Bruker system. The mice were anesthetized with continuously inhaled isoflurane (1.5–2%), respiration was monitored and temperature maintained at 37°C. Following a scout image, axial $T_1$, $T_2$, and $T_2$* weighted imaging were acquired using the following parameters: FOV=3x3 cm, Matrix =128x128, slice thickness =1 mm, and NEX =4, $T_1$ Spin Echo (TR/TE=500/6.5 ms), $T_2$ Fast Spin Echo : (TR/TE=2500/11 to 176 ms with echo spacing =11 ms, Rare factor = 4), and $T_2$* Multi Gradient Multi Echo: ((TR/TE=1500/2.8 to 70.8 ms with echo spacing =4 ms, flip Angle = 20º). Image processing and data analysis were performed in tumors on a voxel-by-voxel basis. $T_2$ and $T_2$* relaxations time were calculated using ParaVision 5.0 package from each imaging session, across all subjects. At the end of the study; tumors were collected and frozen for histologic processing. Murine macrophages were detected using a rat anti-human F4/80 antibody (clone CI:A3-1, AbD Serotec)Following F4/80 immunostaining, slides were stained with Prussian blue (Polyscience Inc) for presence of ferric iron and counterstained with nuclear fast red.

**Results:** MRI data showed differences in tumor signal intensity after USPIO administration compared to baseline. In week 1, $R_2$* increased significantly at 24h compared to baseline (W1-0h: 75.4±5.9, W1-24h: 101.8±9.2; $p<0.05$), no significant differences were observed for $R_2$ values (W1-0h: 19.5±1, W1-24h: 22.63±1.5). In week 2, baseline values for R2 (W2-0h: 20.22±0.4) and $R_2$* values (W2-0h: 80.44±2.4) were similar to baseline values during week 1, suggesting a wash out of iron from the tumor. In addition; both $R_2$ (W2-24h: 33±0.8) and $R_2$* (W2-24h: 185.7±5.4) increased significantly at 24h compared to baseline ($p<0.001$). IHC confirmed co-localization of Ferumoxytol (iron) and F4/80 macrophages.

**Conclusion:** These results suggest that ferumoxytol-enhanced MRI can be used to detect macrophages in CHL-1 melanoma cell derived xenograft tumors. Ferumoxytol was washed out the tumor after one week of 0.5 mmol Fe/kg injection, reflecting the potential to use ferumoxytol-enhanced MRI on a weekly basis to serially monitor response to drug therapies aimed at increasing cellular phagocytosis. Finally, the lower dose of ferumoxytol may be optimal as there will be greater potential to detect increased cellular iron uptake by scavenging macrophage in the tumor.

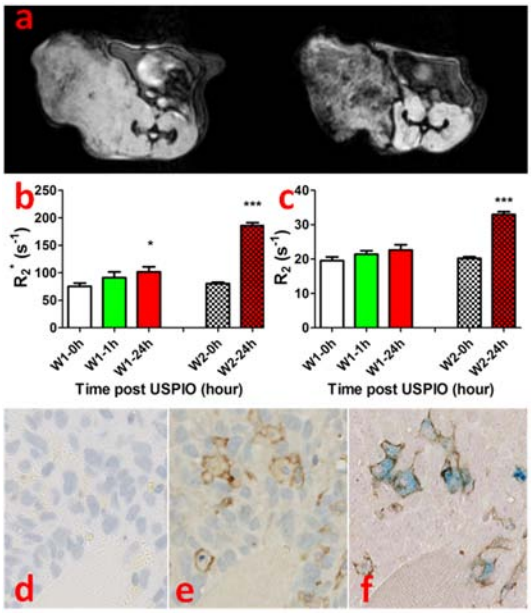


**Figure 1** Axial in-vivo MRI of ferumoxytol uptake in melanoma tumor (a); $T_2$*-weighted images at baseline (precontrast-left panel) and 24 hours after administration of 1 mmol [Fe]/kg ferumoxytol (right panel). Changes in $R_2$* (b) was significant for 2 different doses at 24h post contrast; however changes in $R_2$ (c) was significant only for the high dose of ferumoxytol at 24h post contrast. F4/80 IHC (d) and Perl's single (e) and dual staining (f) was indicative of close association cytoplasmic iron staining with a sub population plasma membrane F4/80 positive cells.

**Reference:** (1) Heike E. Daldrup-Link et al, Clin Cancer Res 2011;17:5695-5704 .