Iron-Oxide Driven Decrease in T2 Relaxation Times Correlates with Tumor Associated Macrophages (TAMs) in Postpartum Pregnancy Associated Breast Cancer Xenografts

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Introduction: Breast cancer is the most commonly occurring cancer among women. Postpartum Pregnancy Associated Breast Cancer (PPABC), a variant, arising up to 5 years postpartum, is very aggressive, highly metastatic and affects young women with children in tow[1]. During pregnancy the mammary gland undergoes hypertrophy and involutes postpartum via a process similar to that of wound healing—inflammatory cytokines released by stromal cells recruit fibroblasts and specialized tumor associated macrophages (TAMs)[2]. Studies have shown that the immunological milieu of tumors arising postpartum is rich in T-cell suppressive factors and cells including IL-10+ macrophages[3]. Thus, this physiologic remodeling process produces a microenvironment, which is highly tumorigenic. PPABC epidemiologic studies show that overall survival rates are lower for women diagnosed with PPABC when compared with virgin (nulliparous) counterparts[4]. Currently, there are several therapies aimed at reducing the inflammation associated with postpartum changes including NSAIDs, and other biologic immunomodulators. The gold standard for diagnosis of this type of cancer is highly invasive biopsies, which come at great cost to patients. Recent MRI-based studies have shown a possibility to non-invasively assess TAMs using super-paramagnetic iron-oxide (SPIO) based contrast and T2-MRI[5]. The goal of this study is to develop quantitative T2- and T2*-MRI using injection of commercially available SPIO nanoparticles for imaging TAMs associated with PPABC in order to risk stratify patients, tailor anti-cancer therapies and to monitor their effects on the inflammatory microenvironment.

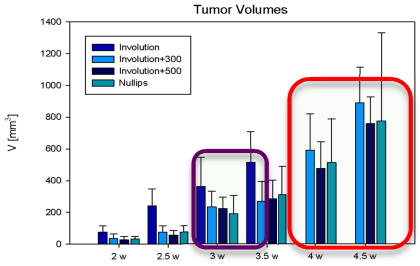

Figure 1. Tumor growth in nulliparous, involution and involution plus ibuprofen treated groups. Cycle 1 is in purple, Cycle 2 is in red.

Methods: For this study 25, eight-week-old female BALB/C mice underwent mammary fat pad injections of isogenic murine mammary tumor cell line D2.A1 and were randomized into four groups, involution (n=4), involution plus 300mg ibuprofen (n=6), involution plus 500mg ibuprofen (n=6) and nulliparous (n=9). After tumors achieved a minimum volume of 200mm^3, the first MRI was performed, a baseline prior to a tail vein injection of 30mg/kg of Feraheme (FDA-approved SPIO nanoparticles) and 24 hours post-injection (Cycle 1). The Cycle 2 MRIs were performed once tumors reached a minimum of 400mm^3, using the same dose of Feraheme. All MRI scans were obtained on anesthetized animals using a 4.7 Tesla Bruker PharmaScan MRI with a 31mm-diamter Bruker volume coil and acquired using Bruker Paravision 4.0.1 software. After obtaining tri-pilots for ROI localization, a conventional rapid acquisition with relaxation enhancement (RARE) proton density (pd) scan was obtained for the volumetric assessment, followed by a multi-slice multi-echo (MSME, 16 echoes) T2w-MRI map and a multi-gradient echo (MGRE, 12 gradient echoes) T2*w-MRI map for the precise calculation of T2 and T2* relaxation times, respectively. All images were analyzed with Bruker Paravision 3.0.2. T2 data for each tumor individually was acquired and then averaged for each group. At the end of MRI scans the mice were sacrificed and tumors and organs harvested for flow cytometry on macrophages and other immunocompetent cells, colorimetric iron quantification and F480/Cd11/Prussian blue immunohistochemistry staining for *ex vivo* correlates.

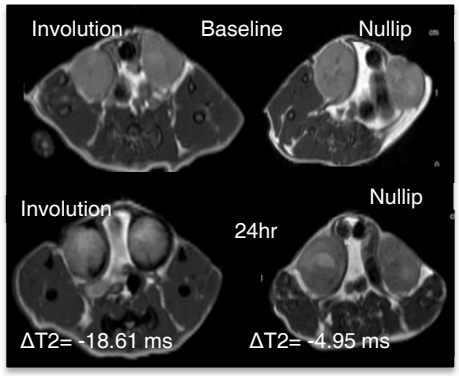

Figure 2 Shows representative T2-weighted MR images for nulliparous and involution mice before (BL) and after injection of SPIO (24 hrs) through Cycle 1.

Results: The involution group showed higher tumor growth rates than nulliparous tumors and, partly, both ibuprofen-treated groups (Figure 1). Volumes for the nullip tumors were highly variable and many of them grew secondary subcutaneous tumors escaping the mammary pad. Only mammary pad tumors are included in the analysis. At the end of Cycle 1, when the tumor size was <400 mm^3, there was a statistically significant drop in T2 relaxation times in the Involution mice after injection of Feraheme. In contrast, the ΔT2 of the nulliparous was practically unchanged and significantly smaller when compared to the involution mice (Figures 2 and 3). At Cycle 1, the presence of macrophages was repeatedly increased in the involution group[3]. The NSAID treatment with ibuprofen had small but significant improvement in ΔT2 when compared to the untreated involution (Figure 3). The involution group also revealed an incomplete recovery of the baseline T2 between Cycle 1 and 2 (ΔT2= -7.03 ms p>0.001) suggesting the presence of residual iron from the first injection, which was retained in highly expressed TAMs. At the end of Cycle 2 (V>400 mm^3) the drop in T2 was identical among the groups (Figure 3) and all four group indicted presence of TAMs when the tumor volume reached >800mm^3 (Figure 2). From the *ex vivo* study, the iron levels were higher in the involution tumors compared to other three groups. Histologically, we were able to co-localize iron deposits (Prussian Blue staining) with TAMs (F480/Cd11 staining) confirming our suspicion that TAMs were in fact imaged.

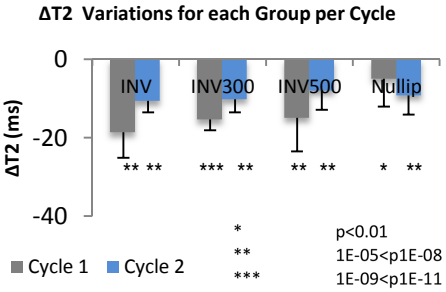

Figure 3. ΔT2 values for Cycle 1 and 2. Involution Cycle 1 ΔT2 was the most statistically significant change (ΔT2=18.61)

Conclusions: Our study shows that Feraheme is an appropriate contrast agent for the assessment of inflammation in PPBAC. In the early stages of tumorigenesis (V<400 mm^3) involution tumors are more inflamed (larger ΔT2, higher TAMs), retain more iron and grow more rapidly than nullip tumors, exhibiting highly aggressive tumor microenvironment and benefiting from anti-inflammatory therapy. Remarkably, as tumor size increased in all groups the physiological relevance of this mouse model declines, the infiltration of TAMs in nullip group increases while SPIO contrast delivery decreases resulting in identical changes in ΔT2 in Cycle 2 among all study groups. Additionally, these data show that Ibuprofen can reduce the ΔT2 changes/TAMs presence of these tumors but in limited extent. By taking advantage of TAM-driven iron metabolism, it is possible to assess the level of inflammation in PPABC, to validate an anti-inflammatory therapy and to bypass the need for *ex vivo* histological assessment of disease progression.

References:

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