Title: OATP1A1 reporter gene-enhanced multimodality imaging of triple negative breast cancer in animal models

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Structured Abstract:

Introduction: Clinical trials in oncology have the lowest success rates compared to those focused on other diseases (Hay et al 2014 Nature Biotechnol). This daunting pattern is unsustainable and investigators must reassess approaches towards translating discovery into clinical impact (Begley and Ellis 2012 Nature). Notwithstanding the complexity of cancer, limitations of preclinical research tools are considered a principal cause (Errington et al 2014 eLife). To address these limitations, our team is developing a highly-resolved and spatially-informative in vivo viability assay based on a magnetic resonance imaging (MRI) reporter gene from the Organic Anion Transporting Polypeptide 1 (OATP1) family. We also demonstrate that OATP1 concomitantly enhances bioluminescence imaging (BLI) signals from the luciferase reporter for improved detection of smaller and/or deeper lesions.

Methods: Human (MDA-MB-231) and murine (4T1) triple negative breast cancer (TNBC) cells were transduced first with a lentiviral vector co-expressing tdTomato and firefly luciferase, followed by a second vector co-expressing zsGreen and OATP1. Cells were characterized via immunofluorescence and growth assays to certify retention of pre-transduction phenotypes. Human and murine luciferase/OATP1-expressing or control luciferase-expressing cells were implanted into mammary fat pads of female (Nu/Nu) mice (n=4; 16 total). Imaging was acquired weekly to track tumour growth. BLI was performed using an In Vivo Imaging System (IVIS; Perkin Elmer), and pre- and post-Gd-EOB-DTPA-enhanced MR images were acquired over 4 weeks on a 3T system. Analysis of variance (ANOVA) tests were used to calculate significance.

Results: Immunofluorescence staining confirmed OATP1 expression and growth assays demonstrated no difference in metabolic activity between OATP1-positive and -negative populations. A significant 4.4-fold increase in BLI output was displayed by human and murine TNBC tumours engineered with luciferase/OATP1 relative to control tumours expressing luciferase alone ($p<0.0001$). A 2.3-fold increase in MR signal intensity was observed by OATP1-expressing tumours, following administration of 0.1 mmol/kg Gd-EOB-DTPA, when compared to control tumours ($p<0.0001$). As tumours grew, intratumoural heterogeneity emerged in the form of enhancing and non-enhancing volumes within post-contrast images of OATP1 tumours. Qualitatively, enhanced volumes mirrored areas of viable cells on histology.

Discussion: Future work focuses on calculating the congruency between volumes of tumour enhancement on MR images and areas of viable engineered TNBC cells on histology. If the OATP1 system proves effective, dissemination of this new tool will pave the path toward molecular imaging of viable cancer cells with combined high spatial resolution, high sensitivity and 3D information, thereby allowing for a holistic, quantitative, and functional assessment of candidate cancer therapies.