Title: A clinically-relevant photoacoustic imaging reporter gene system using indocyanine green

Trainee Name: Nivin Nyström

Supervisor(s): Dr. John Ronald, Dr. Timothy Scholl

Structured Abstract:

Introduction: Photoacoustic imaging (PAI) combines optical contrast with the resolution and depth-detection of ultrasound and is increasingly being utilized for medical imaging in patients. PAI reporter genes would allow for monitoring of cell and gene therapies, but current reporters have immunogenicity and/or toxicity concerns that may limit clinical translation. Here we report a novel PAI reporter gene system employing the ability of human organic anion transporting polypeptide 1b3 (Oatp1b3) to take up the clinical dye indocyanine green (ICG) into cells. The objective of this study was to assess the feasibility of this PAI reporter gene system for visualizing engineered cells implanted in mice.

Methods: Human cells (MDA-MB-231) were engineered with lentivirus to generate Oatp1b3-expressing cells. Control and OATP1B3 cells were incubated with or without 35 μg/ml ICG, washed, and imaged via an optical scanner (780-nm excitation, 845-nm emission), followed by PAI (780-nm excitation) on a custom-built system (n=3). Mammary fat pads of mice were implanted with Control (n=6) or OATP1B3 (n=5) cells. Mice were imaged via FLI as described above, and via spectral PAI (680-970 nm, $\Delta\lambda$=5 nm) before and 24-hours after injecting 8 mg/kg ICG. One-way Analysis of Variance (ANOVA) was performed followed by Tukey's post-hoc multiple comparisons using Graphpad Prism software (Version 7.00 for Mac OS X, GraphPad Software Inc., La Jolla, California, United States, www.graphpad.com). For all tests, a nominal p-value less than 0.05 was considered statistically significant.

Results: Photoacoustic contrast-to-noise ratio (CNR; arbitrary units, a.u.) was significantly increased for OATP1B3 cells incubated with ICG (2.7-fold; $p<0.05$), relative to CNR of ICG-incubated Control cells and all untreated controls. Control cells incubated with ICG did not exhibit a significant difference in CNR relative to untreated control cells. In mice, PAI signals from OATP1B3 tumors (n=5) relative to Control tumours (n=6) were not significantly different prior to ICG administration. However, PAI signals increased 2.3-fold ($p<0.05$) in mice with OATP1B3 tumours compared to Control tumours 24-hours after ICG administration.

Discussion: In this study we establish OATP1B3 and ICG as a novel reporter gene-probe system for in vivo PAI of engineered cell populations. Our system generates a distinct near-infrared (NIR) peak via PAI specifically by engineered cells, high CNR relative to surrounding signals, and low toxicity to the biological system, while retaining benefits such as relative affordability, rapid image acquisition, portability, and safety. Future work focuses on further characterizing this reporter gene system for clinically-relevant modalities such as NIR spectroscopy (NIRS) and fluorescence endoscopy. We expect that this PAI reporter system will be highly valuable for tracking of gene- and cell-based therapies at both preclinical and clinical stages.