DUAL FLIM/PLIM IMAGING IDENTIFIES HYPOXIC REGIONS RESISTANT TO PI3K-PATHWAY TARGETED THERAPIES IN PANCREATIC CANCER

James R.W. Conway[1], Sean C. Warren[1], Robert F. Shearer[1], Monica Killen[1], David Herrmann[1], Astrid Magenau[1], David R. Croucher[1], Yingxiao Wang[3], Owen J. Sansom[2], Marina Pajic[1], Jody J. Haigh[4], Jennifer P. Morton[2] and Paul Timpson[1]

[1]Garvan Institute of Medical Research and Kinghorn Cancer Centre, Cancer Research Program, St. Vincent's Clinical School, Faculty of Medicine, University of New South Wales, NSW, Australia; [2]The Beatson Institute for Cancer Research, Glasgow G61 1BD, UK; [3]Department of Bioengineering, University of Illinois, Urbana-Champaign, Urbana, Illinois, United States of America; [4]Division of Blood Cancers, Monash University, Victoria, Australia

Email: j.conway@garvan.org.au

KEY WORDS: Intravital FLIM-FRET, Phosphorescence Lifetime Imaging Microscopy (PLIM), oxygen-sensitive nanoparticles, drug resistance, biosensors, hypoxia

ABSTRACT: By applying novel intravital imaging techniques to dynamically monitor pathway activity upon therapeutic inhibition, we are assessing PI3K-pathway targeted therapeutic resistance within the hypoxic microenvironment of pancreatic ductal adenocarcinoma (PDAC) [1]. Hypoxia is a negative prognostic factor in PDAC, known to increase radioresistance, chemoresistance, angiogenesis and metastasis. Here we demonstrate pronounced resistance in hypoxia to three clinically relevant inhibitors of the PI3K pathway; Rapamycin (mTORC1 inhibitor), NVP-BEZ235 (dual PI3K/mTORC1 inhibitor) and AZD2014 (mTORC1/2 inhibitor) [2]. Further to the clinical problem, we have mapped tumour hypoxia by both immunofluorescence and phosphorescence lifetime (PLIM) imaging techniques and here have shown that these hypoxic regions move sproadically around the tumour. Using advanced Akt Förster Resonance Energy Transfer (FRET) biosensors and state-of-the-art multiphoton imaging, we will demonstrate improved efficacy for dual PI3K/mTOR inhibitors against this hypoxia-induced resistance.

Within our well-established mouse models of PDAC, we have expressed an optimised Akt-FRET biosensor [3]. To date, *in vitro* modeling has involved three-dimensional invasion/proliferation assessment using organotypic assays and assessment of drug kinetics through live cell fluorescence lifetime (FLIM)-FRET imaging and standard molecular techniques. Progressing to *in vivo* intravital imaging, we are dynamically monitoring the therapeutic effect of targeting the PI3K–Akt–mTOR signaling axis within the hypoxic microenvironment, using novel dual FLIM/PLIM imaging techniques. Here we are able to assess novel combination therapies using hypoxia-activated pro-drugs (i.e. TH-302) and angiogenics, with our dual PI3K pathway inhibitors, to improve treatment efficacy [4]. Further to our *in vivo* fidelity, we have developed a novel Akt-FRET biosensor mouse for real time analysis of drug kinetics in the native disease state.

[1] J.R.W. Conway, et al, "Developments in preclinical cancer imaging: innovating the discovery of therapeutics". *Nat Rev Cancer*. **14**(5): p. 314-28 (2014).

[2] D.C. Morran, et al., "Targeting mTOR dependency in pancreatic cancer". *Gut* (2014).

[3] N. Komatsu, et al., "Development of an optimized backbone of FRET biosensors for kinases and GTPases". *Mol Biol Cell*. **22**(23): p. 4647-56 (2011).

[4] J.D. Sun, et al., "Combination treatment with hypoxia-activated prodrug evofosfamide (TH-302) and mTOR inhibitors results in enhanced antitumor efficacy in preclinical renal cell carcinoma models". *Am J Cancer Res*, **5**(7): p. 2139-55 (2015).