Name of Institution: University of Sydney

Project Title: *Treating Pancreatic Cancer with FXYD3 Peptide Analogues*

Principal Investigator: Prof Helge Rasmussen

Grant: Round 2, Innovation Grant

Background:

Prof Rasmussen and his team made a protein, FXYD3, being a molecular tool used to study the sodium-potassium pump. This pump moves sodium out of cells and potassium in the opposite direction, into cells. The differences in concentrations of sodium and potassium inside relative to their concentration outside caused by the pump is critical for cell survival.

When using the FXYD3-derived tool Prof Rasmussen found that normal FXYD proteins protect sodium-potassium pump function against oxidative stress.

In some cancers, including pancreatic cancer, FXYD3 is present in much greater abundance ("overexpressed") than in non-cancer cells. Prof Rasmussen and his team developed a much shorter version (FXYD3-pep) that has similar properties to the full-length modified FXYD3 protein but is easier and cheaper to make.

The aim of the Project was to determine to what extent FXYD3-pep can increase effects of chemotherapy and explore the mechanism by which such an increase occurs.

The Research:

A brief summary of the most important research findings from the Project made by Prof Rasmussen:

- FXYD3 by itself has little effect on cancer alone but is highly effective in amplifying effects of treatment.
- FXYD3 augments cytotoxicity of cancer treatment up ~10-fold against pancreatic cancer cells overexpressing FXYD3 but not against cells without such overexpression.
- FXYD3-pep is readily taken up by cells and is effective in micromolar concentrations. This is expected to increase cure rates, even at lower dose levels of anticancer drugs and perhaps avoiding cardiac toxicity that often limit doses that can be safely given.

Prof Rasmussen performed *in vivo* studies regarding FXYD3-pep

- Prof Rasmussen established a new collaboration with Prof. Marina Pajic (*A Principal Investigator from a Round 1 Innovation Grant awarded by the Avner Pancreatic Cancer Foundation*) from the Garvan Institute of Medical Research.
- Xenograft pancreatic tumour tissue microarrays were obtained from Prof Pajic's laboratory and screening for FXYD3 expression in these microarrays is currently in progress.
- Prof Rasmussen has completed *in vitro* dose response studies for the optimal concentration of Gemcitabine and FXYD3 decoy.

Prof Rasmussen performed mechanistic studies during inactive periods of *in vivo* studies (waiting for tumours to grow)

Remarkably, Prof Rasmussen found the presence and absence of FXYD3-pep dramatically affected the concentration of an inhibitor where the response (or binding) is reduced by half (IC50 values) of doxorubicin in pancreatic cancer cells approximately four times more without FXYD3-pep compared to with FXYD3-pep after incubation for 48 hours.

Prof Rasmussen's study indicated that the sodium/potassium pump is not the target for FXYD3-pep.

The distribution of FXYD3-pep suggests an intracellular effect rather than an effect on membrane ion transport. This is important because an effect on membrane ion transport would have been expected to affect non-cancer cells and hence have a low therapeutic index.

The FXYD3 gene is linked to the tumour suppressor p53 that has activation of Glutathione *S*-transferase Pi and protection of cells against oxidative stress as one of its multiple roles and is itself regulated by oxidative stress. Tumour suppressor p53 is a tumor suppressor gene and its activity stops the formation of tumors. Glutathione *S*-transferase Pi (GSTπ) is a marker protein in many cancers and high levels are linked to drug resistance.

Prof Rasmussen's research showed reducing FXYD3 function up-regulates p53 and markedly reduces GSTπ expression.

The Impact:

As a result of this Project funded by the Avner Pancreatic Cancer Foundation:

Prof Rasmussen is currently preparing a submission to the National Foundation for Medical Research and Innovation. His application for funding incorporates data produced by the Project funded by the Avner Pancreatic Cancer Foundation. The proposed project will focus on additional studies regarding FXYD3-pep augmentation of chemotherapy and augmentation of effects of radiotherapy against patient-derived pancreatic cancers expressed in mice. Prof Rasmussen and his team have a long-term objective to develop a lead-compound that will ultimately be a clinically useful peptide-drug.

Prof Rasmussen was invited as a plenary speaker to present *Silencing FXYD3 Protein in Human Pancreatic Cancer Cells Enhances Cytotoxic Effect of Doxorubicin* at the 15th International Conference on Na,K-ATPase and Related Transport ATPases, Japan 2017.

Feedback provided by Prof Rasmussen

Funding from the Avner Pancreatic Cancer Foundation has allowed us to explore a very unconventional but promising approach to treatment never previously considered.