Bosch Institute

Translational Workshop

21 March 2013
New Law School Annexe SR 342
Program and Abstracts, Translational Workshop, March 21, 2013

Bosch Institute Translational Meeting
21st March 2013

PROGRAM

12.00 pm  Translating technology for human elastic tissue repair.
          *Anthony Weiss*

12.30 pm  A completely new approach to diagnosing prostate cancer
          *Vicki Velonas, Steve Assinder, Henry Woo, Joshua Ho and Cris dos Remedios*

Lunch:     1.00 pm  Lunch will be served at the Law School

1.30 pm  L-proline and LIF supplementation for cultured human embryos – Improving embryo health and birth rates from assisted reproduction
          *Michael Morris, Melissa Lee and Margot Day*

2.00 pm  Adhesion molecules and red blood cells: a sticky problem.
          *Angeles Sanchez-Perez and Stuart Fraser*

2.30 pm  The cellular uptake and interaction of novel anti-cancer iron chelators with human serum albumin
          *Danuta S. Kalinowski and Angelica Merlot*

Afternoon tea: 3.00 pm

3.30 pm  A randomized, double-blind, placebo controlled trial on the use of a vitamin D-like compound to enhance protection from UV damage
          *Eric Song, Clare Gordon-Thomson, Louise Cole, Gary Halliday, Vivienne Reeve, Rebecca S Mason*

4.00 pm  Role of SAA in promoting endothelial activation
          *Paul K. Witting and Saul Benedict Freedman*

4.30 pm  Commercial development of novel chemotherapeutics that demonstrate broad and specific anti-cancer activity
          *David B. Lovejoy, Danae M. Sharp, Nicole Seebacher, Peyman Obeidy, Tom Pritchard, Christian Stefani, Maram T. Basha, Phillip C. Sharpe, Patric J. Jansson, Danuta S. Kalinowski, Paul V. Bernhardt and Des R. Richardson*

5.00 pm  Roundtable on future of TGIA Program - refreshments and finger food served
TGIA Recipients: Day & Morris

Title:
L-proline and LIF supplementation for cultured human embryos - Improving embryo health and birth rates from assisted reproduction

Authors:
Michael Morris, Melissa Lee and Margot Day

Abstract:
Assisted reproduction technologies (ART) account for approximately 3% of births in Australia. While success rates of oocyte fertilisation are high, only 17% of treatment cycles result in live birth. In an attempt to improve pregnancy rates, there is an increasing trend to culture embryos for longer periods and to transfer embryos back into the mother at the blastocyst stage. This is based on the assumption that any embryos that develop into a blastocyst must be of higher quality. However, it is clear from mammalian models of human development that the culture environment can cause significant alterations in gene expression, epigenetic status, metabolism, and cell proliferation. These alterations can cause developmental block or retardation of embryo development while in culture, loss of embryo viability after transfer, and be detrimental to fetal development. Media currently used for culture of human preimplantation embryos during assisted reproduction are based on media that allow successful in vitro development of mouse embryos from the zygote to the blastocyst stage. These media are essentially a chemically defined saline solution containing metabolic substrates (lactate, pyruvate and glucose), serum albumin and, in some cases, a mixture of non-essential and or essential amino acids. We are currently exploring the effect of supplementation of simple chemically defined medium with L-proline and LIF on development and health of embryos.

Bio:
Margot Day is a Senior Lecturer in the Discipline of Physiology at the University of Sydney and heads the Laboratory of Developmental Physiology. She graduated with Honours and a PhD in Physiology from the University of Sydney. She was a post-doctoral Fellow at the University of Cambridge, UK, funded by an NHMRC CJ Martin Fellowship, before returning to the Discipline of Physiology at the University of Sydney. Her laboratory studies the physiological processes involved in fertilization and proliferation of the cells in the preimplantation embryo and uses a range of molecular, electrophysiological and live cell imaging techniques. Her current research is aimed at understanding the impact of the in vitro culture environment on pre-implantation embryonic development in order to improve human reproductive outcomes.
TGIA Grantee: dos Remedios

Title:
A Completely New Approach to Diagnosing Prostate Cancer

Authors:
Vicki Velonas, Steve Assinder, Henry Woo, Joshua Ho and Cris dos Remedios.

Abstract:

Background - Leukocyte Antibody Microarrays: All cancers create a specific and identifiable inflammatory response by the cellular immune system. When cells such as peripheral blood mononuclear cells are isolated and applied to an extensive 2-D microarray of selected antibodies, the resulting pattern of immobilized leukocytes represents a virtual immunophenotype, i.e. the pattern is unique to the type of cancer or the cause of the inflammation. Here we reveal data that show considerable promise for the diagnosis of prostate cancer using just a teaspoonful of whole venous blood.

Background – Prostate Cancer Detection: The Prostate Specific Antigen (PSA) test is the current, but now flawed, diagnostic method for the detection of prostate cancer. It suffers from both poor sensitivity and poor specificity, producing unacceptably high levels of false negatives and false positives. Our test is based on an array of Abs directed against cluster of differentiation antigens. It consists of 100-150 antibodies directed against cluster of differentiation antibodies immobilized in a two-dimensional array on a nitrocellulose-coated glass slide.

Results: When the mononuclear cells from 20 prostate cancer patients were arrayed on these slides and the data analysed, we found we could obtain an Area Under the Receiver Operator Curve (AUROC) of 0.79. This promising result will be repeated on a larger population and we are on track to extend the test to a further 100 patients. We expect to be able to improve on this result by adding 40 new antibodies directed against prostate cancer epitopes as well as new targets designed to specifically identify surface proteins related to prostate cancer. We expect these improvements with further increase the AUROC result and make it an attractive and simple diagnostic tool for identifying prostate cancer.

Bio:

Academic and Scientific Positions:
- Professor of Anatomy and Biophysics, Bosch Institute, Discipline of Anatomy & Histology, University of Sydney 2000-present.
- Associate Professor, Department of Anatomy, University of Sydney 1980-2000
- US Biophysical Society: Member of the Council (1997-2003), Member of the Executive (2004-2005). I serve on several of its Committees.
- Former President of the Australian Society for Biophysics.
- Director, Institute for Biomedical Research, University of Sydney (1998-2000).
- Associate Dean, Faculty of Medicine, University of Sydney (1999-2002).
- Member of the Scientific Advisor Board, Medsaic Pty Ltd. (2005-2011).


Inventor: I hold several Australian, US and European patents on three patents (e.g. CG dos Remedios, AR Cooke, 2004, Biomolecular toxicity assay, European Patent 1292703).
TGIA Grantee: Fraser

Title:
Adhesion molecules and red blood cells: a sticky problem.

Authors:
Angeles Sanchez-Perez and Stuart Fraser

Abstract:
Paradoxically, patients with diseases of red blood cells or erythrocytes, often die from vascular disease such as thrombosis, stroke or cardiac infarct. In some cases, this can be ascribed to the unusual shape of the diseased erythrocytes, as seen in sickle cell anaemia. However, we have recently found that erythropoiesis or red blood cell production, in mouse models of haematological disease is highly abnormal and leads to abnormal perdurance of adhesion molecule expression on erythrocytes. We are currently exploring the processes that lead to this abnormal adhesiveness of diseased erythrocytes and assessing whether there is a link between up-regulation of adhesion molecule expression and cardiovascular disease. This may serve as a prognostic indicator of potentially fatal vascular disease in patients with red blood cell disorders.

Bio:
Stuart Fraser completed his undergraduate studies in Immunology and Pathology at Monash University and his Ph.D in Biochemistry at the University of Hong Kong. During his postdoctoral studies in Kyoto University, Japan, his interest in the development biology grew. Using the mouse embryo and embryonic stem (ES) cells as model systems, Dr. Fraser studied the processes that control the formation of the mesoderm, and from this germlayer, the generation of the blood and endothelial lineages. These studies expanded during the six years he was Assistant Professor at the Mount Sinai School of Medicine in New York City. Here, he established a number of useful transgenic mouse models to follow blood and blood vessel development during embryogenesis. It was here that he also expanded his studies to investigate dysfunctional red blood cell production in human disease. Dr. Fraser joined the Discipline of Physiology, University of Sydney, in April of this year as Sesquicentennial Lecturer in Molecular Embryology to establish the Laboratory of Blood Cell Development.
TGIA Recipient: Mason

Title:
A randomized, double-blind, placebo controlled trial on the use of a vitamin D-like compound to enhance protection from UV damage

Authors:
Eric Song, Clare Gordon-Thomson, Louise Cole, Gary Halliday, Vivienne Reeve, Rebecca S Mason

Abstract:
Our group and others have reported that the active vitamin D hormone, 1,25-dihydroxyvitamin D, also known as calcitriol, and other vitamin D analogs, reduce several types of UV-induced DNA damage in human and animal studies and reduce photocarcinogenesis in mice. These data suggest that calcitriol or a related compound might be usefully incorporated in a sunscreen or after-sun preparation to reduce the DNA damage of UV that does penetrate into skin. Vitamin D compounds, however, are expensive to synthesize and are relatively unstable. Using molecular modeling, vitamin D-like compounds have been identified which might be predicted to act like calcitriol in our photoprotection studies. In pre-clinical studies, we showed that this was indeed the case. The current clinical trial was designed to test whether one of these agents reduced DNA damage after UV irradiation in human volunteers. The study was approved by the Sydney South West Area Health Service and the University of Sydney Human Ethics Committees. Ten volunteers, recruited from advertisements, were subjected to solar-simulated radiation on the lower back with an Oriel 1000W xenon arc lamp, to determine minimal erythematic doses (MED - the amount of UV that just produces faint redness), in a range of skin types. A further 6 volunteers were subjected to approximately 2 MED on a template with 4 areas marked on the lower back. One region was shielded, while the other regions were treated topically immediately after irradiation with either vehicle (ethanol:water: propylene glycol 2:1:1), calcitriol or the vitamin D-like compound in vehicle. Punch biopsies were taken after various times 0.5-3h and stained for thymine dimers, oxidative DNA damage (8-oxo-7,8-dihydro-2-deoxyguanosine) or nitrosative damage (8-nitro-guanosine). From these preliminary data, a time of 2h post-UV for the biopsies was selected for the trial patients. Seven volunteers were subjected to a similar protocol as described above, except that the treatment solutions were prepared and labelled separately, so that their identity was not known to patient or doctor (Song) who applied them and processed the samples. As further analyses are still ongoing, the study has not yet been unblinded. Nevertheless, inspection of the data on DNA damage, still unblinded, clearly show, for each subject, that there are increases in the 3 types of DNA damage in all the UV irradiated areas, with one treatment clearly showing higher damage scores than the other 2 treatments. This is likely to indicate that topical application of either the vitamin D-like compound or calcitriol, even immediately after irradiation, leads to a rapid and substantial reduction in several types of potentially mutagenic DNA damage. Further studies incorporating the vitamin D-like compound into a sunscreen preparation (with the sunscreen applied at a much lower concentration than normally recommended) are planned.

Bio:
Rebecca Mason, a medical graduate from Sydney University, has research interests in vitamin D, bone and skin. She is on the Editorial Board of the Journal of Bone and Mineral Research and the journal Endocrinology. She
has been a member of the Cancer Councils of Australia Working Party on Sun and Health and consults on vitamin D with these groups, was a member of the International Commission on Illumination’s technical committee on Sunlight, Health and Vitamin D (reported in 2011) and will serve on a new technical committee for the Commission in 2013. In 2009, she received an award from the 14th International Workshop on Vitamin D for “career contributions to vitamin D research” and now is on the Executive Committee for a new series of International Vitamin D workshops. She is Head of Physiology and Deputy Director of the Bosch Institute, University of Sydney, a Board member of Osteoporosis Australia and Immediate Past President of the ANZ Bone and Mineral Society.
Title:
The cellular uptake and interaction of novel anti-cancer iron chelators with human serum albumin

Authors:
Danuta S. Kalinowski, Angelica Merlot, Namfon Pantarat, Sharleen Menezes, Muni Doddareddy, David Hibbs, Des R. Richardson

Abstract
Iron chelators represent a novel treatment avenue to target tumour cells, which have increased requirements for iron due to their rapid proliferation. Importantly, in vivo studies and clinical trials have confirmed the potential of thiosemicarbazone iron chelators as potent anti-cancer agents. For example, the chelator, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT), demonstrated potent anti-proliferative activity both in vitro and in vivo. Dp44mT mediates its anti-cancer activity via a number of mechanisms, including the formation of redox-active iron complexes that result in reactive oxygen species generation. However, the mechanism involved in the cellular entry of Dp44mT to induce cell death is unclear. Thus, Dp44mT was radiolabelled with 14C in order to assess the mechanisms involved in its cellular uptake in human SK-N-MC neuroepithelioma cells. Uptake studies were carried out in comparison to the more lipophilic chelator, 14C-2-benzoylpyridine 4-ethyl-3-thiosemicarbazone (14C-Bp4eT). The cellular uptake of 14C-Bp4eT was found to occur via passive diffusion. In contrast, the temperature-dependent and saturable uptake of 14C-Dp44mT suggested a receptor-mediated process. The uptake of 14C-Dp44mT decreased in the presence of increasing concentrations of its unlabelled counterpart, Dp44mT, and also suggested the involvement of a saturable transport system. The uptake of 14C-Dp44mT was unaffected in the presence of increasing concentrations of unlabelled Bp4eT. Importantly, the uptake of 14C-Dp44mT was found to markedly (p<0.01) increase in the presence of human serum albumin (HSA; 40 mg/mL), a protein that is well known to bind drugs. This effect was inhibited in the presence of excess HSA, indicating that a HSA receptor may be involved. The enhanced uptake of 14C-Dp44mT observed in the presence of HSA was specific to this protein and was found to occur in six different cell types, suggesting that this mechanism was not cell type specific. Subsequent drug-protein binding experiments suggested that Dp44mT binds directly to HSA at Sudlow’s Site I. In conclusion, these studies suggest that the ability of Dp44mT to bind to HSA results in enhanced uptake into human cancer cells by a saturable, receptor-mediated process, potentially involving an HSA receptor.

Bio:
Dr Danuta S. Kalinowski completed her undergraduate degree in Chemistry at Macquarie University, Sydney. She received her doctorate in Medicine in 2007 at The University of Sydney examining the design, development and medicinal chemistry of novel iron chelators as anti-cancer agents. Dr Kalinowski has held funding from the NHMRC (2009-2011) and the Cancer Institute NSW (CINSW; 2009, 2011). Additionally, she received a Cancer Institute NSW Early Career Development Fellowship (2009-2012) and is currently a Research Fellow at The University of Sydney. Dr Kalinowski currently holds funding from the NHMRC (2013-2015) and her research involves implementing albumin as a pharmacological carrier of novel anti-cancer agents.
TGIA Recipient: Richardson

Title
Commercial Development of Novel Chemotherapeutics that Demonstrate Broad and Specific Anti-Cancer Activity

Authors
David B. Lovejoy,† Danae M. Sharp,† Nicole Seebacher,§ Peyman Obeidy,† Tom Pritchard,† Christian Stefani,§ Maram T. Basha,† Phillip C. Sharpe,§ Patric J. Jansson,† Danuta S. Kalinowski,§ Paul V. Bernhardt† and Des R. Richardson†

† Iron Metabolism and Chelation Program, Department of Pathology, University of Sydney, Sydney, New South Wales, 2006, Australia.
‡ School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Qld 4072 Australia.

Abstract
We developed a series of second generation di-2-pyridyl ketone thiosemicarbazone (DpT) and 2-benzoylpyridine thiosemicarbazone (BpT) ligands in an effort to improve the efficacy and safety profile of these potential anti-tumor agents. Two novel DpT analogues, Dp4e4mT and DpC, exhibited pronounced and selective activity against human lung cancer xenografts in vivo via both the intravenous and oral routes. Importantly, these analogues did not induce the cardiotoxicity observed at high non-optimal doses of the first generation DpT analogue, Dp44mT, and exhibited a substantially improved safety profile in vivo. The Cu(II) complexes of these ligands exhibited potent anti-proliferative activity having redox potentials in a range accessible to biological reductants. The activity of the copper complexes of Dp4e4mT and DpC against lung cancer cells was synergistic in combination with either gemcitabine or cisplatin, being more active than the standard combination of these latter two chemotherapeutics.

Published in:

Brief Biography
Dr. Des Richardson B.Sc., M.Sc., Ph.D., D.Sc., FFSc, FRCPath (UK) is Professor of Cancer Cell Biology and an NHMRC Senior Principal Research Fellow at the Department of Pathology, University of Sydney. He has published over 300 articles, chapters, patents and books and is commercially developing a new anti-tumour agent known as DpC in cooperation with Cthulhu Ventures, California, USA.
TGIA Grantee: Weiss

Title:
Translating technology for human elastic tissue repair.

Authors: A. Weiss

Abstract:
Human elastic tissues include skin and blood vessels. Elastin facilitates elasticity, resilience, persistence and dedicated cell interactions. The Weiss Lab is making 3D constructs to repair and augment these and other elastic tissues by leveraging its international leadership in human tropoelastin, synthetic elastin and elastin based therapies. The corresponding technologies are backed by a portfolio of awarded patents, coupled with a blend of fundamental science, applied technologies and translation to clinic. Professor Weiss will provide an overview of these areas.

Bio:
Professor Tony Weiss is the world leader in the science and applications of synthetic human elastic tissue. His lab is revealing Nature's assembly rules to make elastic materials for synthesising and improved healing of skin, arteries and other 3D human tissue components. He is Professor of Biochemistry in the School of Molecular Bioscience, Professor Charles Perkins Centre, and Professor Bosch Institute University of Sydney, and holds an honorary Professorial appointment in the Department of Cardiology, Royal Prince Alfred Hospital. He is the scientific founder of Elastagen Pty Ltd, a clinical stage company which is currently conducting clinical trials on for the repair and augmentation of human skin. His most awards include winning the Australian Innovation Challenge Prize, Sir Zelman Cowen Exchange Fellowship, Fondation des Treilles Scholarship and Pauling Prize Medal. He was recently elected to TERMIS Council and elected a Fellow of the American Institute for Medical and Biological Engineering. He has 21 awarded international patents in multiple jurisdictions and further patents pending. He has multiple grants that include the ARC, NHMRC and NIH USA. He is on four Editorial Boards and was recent BSB national chair of the ARC College of Experts.
Title: Role of SAA in promoting endothelial activation

Authors: Paul K. Witting and Saul Benedict Freedman

Abstract

Hepatic production of the acute phase protein Serum amyloid A (SAA) promptly increases in response to inflammatory conditions. Circulating SAA is biologically active and stimulates pro-inflammatory and pro-thrombotic responses in isolated blood cells such as peripheral blood mononuclear cells. Stimulation of nuclear factor kappa beta (Nfκβ) activation by SAA is in part responsible for its action on different cell types. More recent data indicates that SAA can induce endothelial activation and this can lead to endothelial dysfunction through a mechanism of enhanced oxidative stress. Taken together, these processes likely contribute to a pro-atherogenic action for SAA in the vasculature. It is now understood that chronic accumulation of SAA in vascular lesions occurs at all stages of atherogenesis. Furthermore, accumulating evidence implicates SAA in processes that potentiate atherosclerosis. For example, the over-expression of SAA in apolipoportein E deficient (apoE⁻/⁻) mice has reinforced this point of view by demonstrating an accelerated formation of vascular lesion relative to controls that occurred concomitantly with an increase in the levels of pro-inflammatory cytokines. Despite growing understanding of the chronic inflammatory nature of atherosclerosis, specific anti-inflammatory therapy has yet to be established. Notably, human high-density lipoprotein is able to modulate SAA activity on the vascular endothelium. As a future perspective, the role for HDL as an inhibitor of SAA's pro-atherogenic activity on the vascular endothelium will be discussed in this presentation.

Bio:

Dr Paul Witting is an Associate Professor at The University of Sydney and a career biomedical researcher. He has held ARC (2003-2007) and National Heart Foundation Fellowships (1999-2001) that financed senior research positions at the ANZAC Research Institute (ARI) and University of British Columbia (Canada), respectively. He is presently funded by grants from the ARC and Heart Foundation as well as commercial funding from Servier International, and The Bosch Institute (Translational grant-in-aid). He received his doctorate in 1994 graduating from the School of Chemistry (University of Sydney) where he specialised in redox chemistry. Next, he completed a post-doctoral appointment at The Heart Research Institute with Professor Roland Stocker and then spent 2 years as a Visiting Postdoctoral Fellow in the Biochemical and Molecular Physics Department at the University of British Columbia. His current research focuses on exploring a role for oxidative stress in myocardial and cerebral ischaemia.