24th NSW Stem Cell Network Workshop

Stem Cells and Cancer

Darlington Centre
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Wednesday, April 6th, 2016

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WELCOME

Welcome to the 24th Workshop of the NSW Stem Cell Network

Cancer is a major cause of morbidity and mortality worldwide, so there is a growing need to better understand and treat this group of disease. Stem cell research has not only advanced our knowledge about the mechanisms regulating malignant cell development, but it has also given rise to novel potential cancer treatments. New stem cell concepts are constantly redirecting cancer research and a lot of progress has been made since the first descriptions of cancer stem cells. The present workshop, "*Stem Cells and Cancer*" is dedicated to discussing new findings of this rapidly evolving research field.

We will start by exploring key biological and functional characteristics of stem cells and cancer, including the use of pluripotent stem cells and medical genomics to understand malignancies and mechanisms underpinning drug resistance.

Next, we will discuss therapies. Conventional cancer treatments are suboptimal in many ways. The effectiveness of 'classical' therapies is often limited by drug resistance. Furthermore, traditional antineoplastic agents are not specific to tumour cells, which is far from ideal. In this workshop, we will explore novel and potential cell-based therapies for cancer. Professor Hiroshi Kawamoto (Kyoto University) will present his breakthrough work on tumour antigen-specific T cells derived from induced pluripotent stem cells (iPSCs). These iPSC-derived T cells can potentially be applied to treat cancer. Other promising therapeutic options, such as the use of dendritic cells for cancer immunotherapy and GD2-chimeric antigen receptor (CAR) T-cell therapy for melanoma also will be discussed.

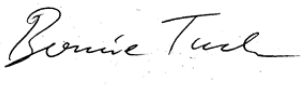
Research on haematopoietic stem cells and cancer has been advancing quickly, so the workshop will explore the most recent information on leukaemia and other blood malignancies. Furthermore, there will be a session focusing on stem cell applications to breast, prostate and testicular cancers.

By gathering key Australian and international experts in stem cells and cancer, this event aims to provide opportunities for cross-disciplinary collaborations and innovative inquiries, leading to more efficient translation of stem cell research. We hope you can take advantage of this unique occasion to discuss the main advances and issues in stem cell and cancer research with fellow leaders in the field.

The 24th NSW Stem Cell Network Workshop would not have been possible without the generous help from our sponsors, speakers and all of you present today. We are truly grateful for your support and contribution.

We also would like to thank Dr Michael Morris, who was of major assistance in planning the Program, and Professor Richard Boyd for facilitating Professor Kawamoto's visit to Australia.

We hope you enjoy the workshop and continue to support the NSW Stem Cell Network at future events!



Dr. Veronica Antas
NSW Stem Cell Network
Manager

Prof. Bernie Tuch
NSW Stem Cell Network
Director

PROGRAM

9:00am	Registration opens/ Light refreshment
9:30am	**Professor Bernie Tuch - NSW Stem Cell Network** *Welcome*
9.35am	**Dr Veronica McCabe - Strategic Research Investment, Cancer Institute NSW** *Opening address*
Session 1	**Overview** **Chair: Dr Michael Morris (University of Sydney)**
9:45am	**Professor Martin Pera (University of Melbourne)** *Pluripotent stem cells: a paradigm for understanding cancer in a developmental context*
10:05am	**Dr Ann-Marie Patch (QIMR Berghofer Medical Research Institute)** *Medical genomics of cancer*
10:25am	**Dr Jenny Wang (University of New South Wales)** *Leukaemia stem cells - drug resistance and targeting*
10:45am	Morning tea
Session 2	**Therapies** **Chair: Dr Janet Macpherson (University of Sydney)**
11:05am	**Professor Hiroshi Kawamoto* (Kyoto University, Japan)** *Cloning and expansion of antigen specific T cells using the iPSC technology: a novel strategy for cancer immunotherapy*
11:25am	**Professor John Rasko, AO (Centenary Institute)** *TBA*
11:45am	**Professor Michael Brown (Royal Adelaide Hospital Cancer Centre)** *GD2-CAR T-cell therapy for melanoma*
12:05pm	**Professor Derek Hart (ANZAC Research Institute)** *An improved dendritic cell vaccine for acute myeloid leukaemia*
12:25pm	Lunch/ Poster session
Session 3	**Blood** **Chair: A/Prof John Pimanda (University of New South Wales)**
1:25pm	**Professor Andrew Elefanty (Murdoch Childrens Research Institute)** *Generation of definitive haematopoietic cells from in vitro differentiated human pluripotent stem cells*
1:45pm	**A/Prof Susie Nilsson (CSIRO)** *Therapeutic targeting and rapid mobilisation of endosteal HSC using a small molecule integrin antagonist*
2:05pm	**A/Professor Paul Ekert (Murdoch Childrens Research Institute)** *The dose-dependent effects of microRNA-155 in leukemia*

PROGRAM

2:25pm	Afternoon tea
Session 4	**Reproductive system** **Chair: A/Professor Stephen Assinder (University of Sydney)**
2:45pm	**Professor Geoff Lindeman (Walter and Eliza Hall Institute)** *Understanding stem cells to get abreast of breast cancer*
3:05pm	**Professor Gail Risbridger (Monash University)** *Stem cells and prostate cancer*
3:25pm	**Professor Kate Loveland (Monash University)** *What influences male germline stem cells and testicular cancer?*
3:45pm	Refreshments / Networking

*Due to medical reasons, Prof Hiroshi Kawamoto could not attend the Workshop. Dr Takuya Maeda (Kyoto University) will present on his behalf.

Professor Martin Pera - University of Melbourne

Professor Martin Pera is Professor of Stem Cell Sciences at the University of Melbourne, the Florey Neuroscience Institute, and the Walter and Eliza Hall Institute for Medical Research**.** He serves as Program Leader for Stem Cells Australia, the Australian Research Council Special Research Initiative in Stem Cell Sciences. Prof. Pera received his BA in English Language and Literature from the College of William and Mary, and his PhD in Pharmacology from George Washington University, and undertook postdoctoral training in the UK at the Institute of Cancer Research and the Imperial Cancer Research Fund. He held independent research positions at the Institute of Cancer Research and the Department of Zoology at Oxford University before joining Monash University in 1996. In 2006 he moved to Los Angeles to take up a position as the Founding Director of the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at the University of Southern California. He returned to Australia in 2011. Prof. Pera's research interests include the cell biology of human pluripotent stem cells, early human development, and germ cell tumours. Pera was among a small number of researchers who pioneered the isolation and characterisation of pluripotent stem cells from human germ cell tumours of the testis, work that provided an important framework for the development of human embryonic stem cells. His laboratory at Monash University was the second in the world to isolate embryonic stem cells from the human blastocyst, and the first to describe their differentiation into somatic cells *in vitro*. His current research is focused on the extrinsic regulation of self-renewal and pluripotency, heterogeneity in pluripotent stem cell populations, and neural specification of pluripotent stem cells. He has provided extensive advice to state, national and international regulatory authorities on the scientific background to human stem cell research. He and his colleagues are currently leading a campaign against the provision of unproven autologous stem cell treatments in Australia. He serves on the Editorial Board of Cell Stem Cell, Stem Cell Reports, Stem Cells, and Stem Cell Research, and on the Steering Committee of the International Stem Cell Initiative. Prof. Pera has been a member of the ISSCR since its inception in 2002. He served on its Standards Committee, and is currently a member of the Audit Committee, and the Chair of the Membership Committee.

Pluripotent stem cells: a paradigm for understanding cancer in a developmental context

Studies begun in the 1950[1]s established teratocarcinomas as a paradigm for understanding cancer stem cells and their place in the context of normal embryonic development. The teratocarcinoma model remains relevant to current concepts of stem cells and cancer. Unravelling how genetic change results in the transformation of normal pluripotent stem cells to a cancer-like phenotype is critical for the application of these cells in regenerative medicine. Finally the use of pluripotent stem cells as a model for human development and its disorders requires a clear understanding of where these cells fit into a developmental paradigm, a question that remains a challenge for the field despite years of study.

Dr Ann-Marie Patch - QIMR Berghofer Medical Research Institute

Dr Ann-Marie Patch is currently a Senior Research Officer within the Medical Genomics group led by Dr Nicola Waddell, at the QIMR Berghofer Medical Research Institute. Her current research focuses on cancer genomics working within large collaborative groups to identify the molecular basis of a variety of cancers including melanoma and mesothelioma. She has a strong research interest in developing methods for understanding the consequences of structural variants in cancer and linking this with the mechanisms of DNA repair. She initially worked on inherited human diseases, after gaining her PhD in 2006, as a bioinformatian in the Royal Devon and Exeter Molecular Genetics Laboratory using next generation sequencing to identify monogenic causes of neonatal diabetes and causal mutations for a broad spectrum of genetic disorders. Cancer genomics has been her focus for the last five years leading the analysis of the ovarian cancer data as part of the Australian ICGC team led by Profs Sean Grimmond and David Bowtell.

Medical genomics of cancer

Genome sequencing has made great advances in the understanding of tumour development and progression. The genome data has been used to classify tumours into significant subtypes, discover driver mutations, identify the mutational processes that underlie tumour development and find alternative therapeutic targets. These are important steps towards ‘personalized medicine’ where the diagnosis, management and treatment of patients are based on the individual’s genomic data. Here I will share some of our key findings from whole genome sequencing of cancer genomes.

Dr Jenny Wang - University of New South Wales

Dr Jenny Wang is Head of the Cancer and Stem Cell Biology Laboratory at CCI/ UNSW. Since returning to Australia in 2011 from Harvard Medical School/Harvard Stem Cell Institute, where she undertook postdoctoral research in Leukaemia Stem Cell Biology (2005-2011), Dr Wang was awarded CINSW Career Development Fellowship in 2012 and Australian Research Council (ARC) Future Fellowship in 2013.

The main focus of her research in the Cancer and Stem Cell Biology Laboratory is to develop novel therapeutic strategies specifically targeting and destroying leukaemia stem cells that are often resistant to commonly used cancer therapies such as radiation therapy and chemotherapy, and that are now believed to be the engine driving the growth of a tumour and the root cause for treatment failure and relapse in blood cancer.

Leukaemia stem cells - drug resistance and targeting

Acute myeloid leukaemia (AML) is a lethal blood cancer. The majority of AML patients experience a recurrence of their cancer after initial treatment (called relapse) and eventually die from their disease. Clinical evidence has supported the important role of leukaemia stem cells in the high relapse rate of AML patients. Genetic and epigenetic abnormalities enable leukaemia stem cells to hijack normal stem cell self-renewal mechanisms that allow leukaemia stem cells to evade therapy and regenerate cancer, leading to relapse. Targeted disruption of abnormal stem cell self-renewal represents a novel therapeutic strategy that could significantly reduce the capacity of a tumour to regenerate itself after treatment and has become a new focus for drug development.

Professor Hiroshi Kawamoto* - Kyoto University

Professor Hiroshi Kawamoto is a researcher of immunology and hematology. He was born in Kyoto, Japan, in 1961. He graduated from the Faculty of Medicine, Kyoto University, in 1986, and worked as a hospital physician for three years. He took his doctor course in Hematology Department of Kyoto University from 1989, and then joined Prof. Katsura's laboratory at the Chest Disease Institute (currently the Institute for Frontier Medical Sciences) from 1994 to 2001 as a visiting researcher, where he started to study early hematopoiesis and T cell development. In 2001, he became an Assistant Professor of Prof. Minato's laboratory in Faculty of Medicine. He was then promoted to a team leader of RIKEN Research Center for Allergy and Immunology in 2002. In RIKEN, in parallel with the basic research, he has started the study on the development of immune cell therapy using regenerated lymphocytes. He moved to Kyoto University in 2012.

Selected publications:

1. Vizcardo R, Masuda K, Yamada D, Ikawa T, Shimizu K, Fujii S-I, Koseki H, Kawamoto H. Regeneration of human tumor antigen-specific T cells from iPS cells derived from mature CD8+ T cells. Cell Stem Cell, 12: 31-36, 2013.

2. Ikawa, T, S Hirose, K Masuda, K Kakugawa, R Satoh, A Shibano-Satoh, R Kominami, Y Katsura, H Kawamoto. An essential developmental checkpoint for production of the T cell lineage. Science. 329: 93-96, 2010.

3. Kawamoto H, T Ikawa, K Masuda, H Wada, and Y Katsura. A map for lineage restriction of progenitors during hematopoiesis: the essence of the myeloid-based model. Immunol Rev. 238:23-36, 2010.

4. Wada H, Masuda K, Satoh R, Kakugawa K, Ikawa, T, Katsura Y, Kawamoto H. Adult T cell progenitors retain myeloid potential. Nature, 452: 768-772, 2008.

Cloning and expansion of antigen specific T cells using the iPSC technology: a novel strategy for cancer immunotherapy

Whereas cytotoxic T lymphocytes (CTLs) represent the most promising therapeutic avenue in cancer immunotherapy, most of the current ongoing trials to utilize CTLs in adoptive are still not effective enough to cure patients, except for cases where invasive pretreatment is give to the recipient or specific antigen receptor gene is transferred to the patient's CTLs. We then came to the idea to apply the induced pluripotent stem cell (iPSC) technology for the cloning and expansion of CTLs. When iPSCs are established from antigen specific T cells (T-iPSCs), these T-iPSCs should inherit rearranged genomic structures of T cell receptor genes, and thus all T cells regenerated from the T-iPSCs should express the same TCR. Since iPSCs can be expanded almost unlimitedly, it is possible to obtain as many fresh CTLs as needed. Along with this idea, we have recently succeeded in regenerating melanoma antigen MART1-specific CTLs from T-iPSCs originally derived from a melanoma patient (Cell Stem Cell, 12: 31, 2013).

While the above approach has been based on the idea of autologous transplantation, we are also applying this method to the allogeneic transplantation settings, in which the T-iPSCs from healthy donors are banked and the regenerated T cells are given to other HLA-matched patients. In this context, we have succeded in establishing iPSCs from CTLs specific for tumor antigen WT1 (Wilm's tumor 1) from healthy volunteers. The regenerated CTLs exhibited very high antigen specific killing activity comparable to the original CTLs. Our study therefore illustrates an approach for the cloning and expansion of functional antigen-specific CTLs that might be applicable in cell-based therapy of cancer.

*Due to medical reasons, Professor Hiroshi could not attend the Workshop. Dr Takuya Maeda (Kyoto University) will present on his behalf.

Professor John Rasko, AO - Centenary Institute

Professor John Rasko, BSc(Med), MBBS(Hons), PhD, MAICD, FFSc(RCPA), FRCPA, FRACP, FAHMS

Professor John Rasko is an Australian pioneer in the application of adult stem cells and genetic therapy. He directs the Department of Cell and Molecular Therapies at Royal Prince Alfred Hospital and heads the Gene and Stem Cell Therapy Program at the Centenary Institute, University of Sydney.

John Rasko is a clinical hematologist, pathologist and scientist with a productive track record in gene and stem cell therapy, experimental haematology and molecular biology. In over 150 publications he has contributed to the understanding of stem cells and haemopoiesis, gene transfer technologies, oncogenesis, human aminoacidurias and non-coding RNAs.

He serves on Hospital, state and national bodies including Chair of GTTAC, Office of the Gene Technology Regulator – responsible for regulating all genetically-modified organisms in Australia - and Chair of the Advisory Committee on Biologicals, Therapeutic Goods Administration. Contributions to scientific organisations include co-founding (2000) and past-President (2003-5) of the Australasian Gene Therapy Society; Vice President, International Society for Cellular Therapy, Australia/New Zealand (2008-12) and established ISCT-Australia; Scientific Advisory Committees and Board member for philanthropic foundations; and several Human Research Ethics Committees. He is a founding Fellow of the Australian Academy of Health and Medical Sciences. He is the recipient of national (RCPA, RACP, ASBMB) and international awards in recognition of his commitment to excellence in medical research, including appointment as an Officer of the Order of Australia.

Abstract was not available at the time of printing.

Professor Michael Brown - Royal Adelaide Hospital Cancer Centre

Professor Michael Brown is Director of the Cancer Clinical Trials Unit at Royal Adelaide Hospital, Adelaide, Australia. He heads its melanoma research program and chairs its fortnightly melanoma multidisciplinary meeting. His particular interests are preclinical and clinical development of chimeric antigen receptor technology and therapeutic arming and targeting of monoclonal antibodies. He trained originally as a dual-diploma clinical and laboratory immunologist before becoming a consultant medical oncologist at the Royal Adelaide Hospital Cancer Centre in 1998. His PhD was in gene therapy and cancer immunotherapy, and his laboratory is situated in the Centre for Cancer Biology, SA Pathology and University of South Australia.

GD2-CAR T-cell therapy for melanoma

Michael P Brown[1,2,3], Wenbo Yu, Susan N Christo, John D Hayball, Eric S. Yvon[4], Gianpietro Dotti[4] Malcolm K Brenner[4], Tessa Gargett[1]

[1]Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide

[2]Cancer Clinical Trials Unit, Royal Adelaide Hospital, Adelaide

[3]Discipline of Medicine, University of Adelaide, Adelaide

[4]Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas, USA

Chimeric Antigen Receptor (CAR) T-cell therapy is an experimental treatment showing great promise for B-cell malignancies but results are more variable in treatment of solid tumours. Metastatic melanoma is a solid tumour in which great treatment advances have been made using either small-molecule inhibitors of the mitogen-activated protein kinase pathway or immune checkpoint inhibitory antibodies, which target T-cell expressed CTLA4 or PD1. Anti-PD1 antibodies, pembrolizumab and nivolumab, have recently been approved and are now the standard of care for metastatic melanoma patients with BRAF-negative disease.

A key correlate of the anti-tumour efficacy of CAR T-cell therapy is the persistence and expansion of CAR T cells within patients. Lack of immunological 'space', functional exhaustion, immune suppression mediated by tumour microenvironments and deletion have all been proposed as mechanisms that may hamper CAR T-cell persistence. Here, we describe the events following activation of third-generation CD3ζ/CD28/OX40 CAR T cells specific for GD2. GD2-specific CAR T cells had highly potent immediate effector functions without evidence of functional exhaustion *in vitro*, although reduced cytokine production reversible by PD1 blockade was observed after longer term culture. Activation-induced cell death of the GD2-specific CAR T cells occurred following repeated antigen stimulation. PD-1 blockade enhanced both CAR T-cell survival and promoted killing of PD-L1-positive tumour cell lines. We present CAR T-cell persistence data from four patients enrolled in the CARPETS phase 1 clinical trial of GD2-specific CAR T cell therapy for metastatic melanoma (ACTRN12613000198729), and characterise the immune phenotype of CAR T cells detectable in the peripheral blood. Small proportions of CAR T cells with central memory or stem cell-like memory phenotypes were found in the infused product and were later identified in post-infusion peripheral blood samples. In all, these data suggest that deletion may also occur *in vivo* and suggest that combination therapy with anti-PD1 antibodies may augment CAR T-cell efficacy and persistence in patients.

Professor Derek Hart - ANZAC Research Institute

Professor Derek Hart, MB, ChB, DPhil, FRACP, FRCPA

Professor Derek Hart has made pioneering contributions to his special interest in dendritic cells (DC), immunotherapy and haematopoietic cell transplants. He is a Rhodes Scholar and RCPA Distinguished Fellow. Prof Hart established the University of Sydney, Dendritic Cell Research (DCR) Group at the ANZAC Research Institute with translational sites at the RPA, Westmead and Concord Hospitals. His human DC research program was the first to clone several CD antigens and define several human DC subsets. The Group continues to define DC cell surface antigens, study DC subset function and novel gene deleted and preclinical humanized mouse models, as it develops diagnostic and therapeutic antibodies to DC.

An improved dendritic cell vaccine for acute myeloid leukaemia

Immune therapy is an effective new modality for treating cancer, particularly when there is good disease control. Therapeutic dendritic cell (DC) vaccination has the potential to generate anti-cancer responses with minimal toxicity. Monocyte-derived DC (Mo-DC) loaded with various forms of tumour associated antigens (TAA) have been used in most clinical trials to date but we emphasized that these *in vitro* manufactured cells are very different to circulating blood DC and clinical data suggests that Mo-DC do not migrate from the injection site. We pioneered the use of monoclonal antibody selection of blood DC as an alternative option and completed a phase I trial of therapeutic vaccination in advanced prostate cancer (J Immunother 2015). We have since manufactured a new human IgG4 chimeric CMRF-56 antibody that can be used to isolate multiple subsets of naturally circulating DC. We have also shown for the first time that blood DC can be loaded efficiently with mRNA encoding TAA (OncoImmunology in press). When compared to Mo-DC, blood DC loaded with *in vitro*-transcribed mRNA resulted in prolonged and superior presentation of translated protein by MHC I molecules, and an increased capacity to migrate to lymph node homing chemokines. There is an urgent need for new therapies for patients with acute myeloid leukaemia (AML). We hypothesized that CMRF -56 immune selected blood DC from AML patients in complete remission after initial chemotherapy would be suitable for therapeutic use. Our work has confirmed that this is possible and shown that CMRF-56+ DC prime functional anti-leukaemia (Wilms tumour 1) T cell responses, following maturation and transfection with mRNA encoding leukaemia antigens. Our initial studies have identified abnormal T cell checkpoint molecule expression in AML patients. Primary CMRF-56+ blood DC may be a superior source of DC for therapeutic vaccination and coincidental checkpoint inhibitor therapy may improve their clinical efficacy.

Professor Andrew Elefanty - Murdoch Childrens Research Institute

Professor Andrew Elefanty trained as a physician and completed a PhD in leukemogenesis at the Walter and Eliza Hall Institute of Medical Research. He subsequently worked on globin gene regulation with Professor Frank Grosveld at the National Institute for Medical Research in Mill Hill, London before returning to the Hall Institute to pursue interests in developmental haematopoiesis and the differentiation of embryonic stem cells.

He moved to Monash University in 2002 with colleague Professor Ed Stanley to initiate studies with human embryonic stem cells. In July 2012, his laboratory relocated to the Murdoch Childrens Research Institute.

Professor Elefanty's work has focused on human pluripotent stem cell differentiation, with a special interest in haematopoietic lineages.

His laboratory aims to generate cells to model blood diseases *in vitro* and for transplantation. The laboratories of Professor Elefanty and Professor Stanley have generated genetically modified human stem cell lines in which lineage-specific fluorescent reporters allow monitoring of differentiation. Professor Elefanty is a National Health and Medical Research Council (NHMRC) Senior Research Fellow.

Generation of definitive haematopoietic cells from in vitro differentiated human pluripotent stem cells

Hematopoietic stem cell (HSC) transplantation reconstitutes the blood cell compartment following myeloablative therapy or for patients with marrow aplasia. Because many patients do not have an optimal matched donor, the provision of HSCs from alternate sources, such as differentiated human pluripotent stem cells (hPSCs), is required. Despite considerable efforts, it has not been possible to efficiently generate repopulating HSCs from PSCs. Based on key roles for *SOX17* in hemogenic endothelium and in the earliest HSCs, and for *RUNX1C* in marking hematopoietic progenitors, we reasoned that reporter lines that marked cells expressing these genes would be valuable for identifying definitive hematopoietic lineages. In initial studies, we found that *RUNX1C* marked a subset of CD34+ cells highly enriched for haematopoietic progenitors that homed to the bone marrow, but did not engraft immunocompromised mouse recipients. Exploring molecular differences between hPSC-derived and cord blood CD34+ cells revealed that the RUNX1C+CD34+ cells failed to express *HOXA* genes. We found that modulating ACTIVIN and WNT signalling, timed to overlap with the peak expression of primitive streak genes, enhanced chromatin accessibility across the *HOXA* cluster and up-regulated *HOXA* expression, effectively providing a 'switch' from primitive to definitive hematopoiesis. This led to the formation of striking SOX17+ vascular structures, which generated RUNX1C+ haematopoietic cells, mimicking aspects of human aorta-gonad-mesonephros (AGM). The *HOXA*-expressing cultures sustained haematopoiesis longer than control cultures, evidenced by the prolonged generation of colony forming cells, which included erythroid precursors that had switched from embryonic to fetal globin expression. Our findings argue that *HOXA* codes established early in differentiation predict cellular potential and provide correct cell patterning for the specification of definitive hematopoietic lineages from hPSCs. Our identification of the relationship between specific signaling events and *HOXA* gene induction represents a significant step towards the generation of transplantable human hematopoietic stem cells from pluripotent stem cells.

A/Professor Susie Nilsson - CSIRO

Associate Professor Susie Nilsson received her Ph.D. in hemopoiesis from Melbourne University. She was made an adjunct Associate Professor at Monash University, Melbourne, Australia in 2006, joining ARMI in 2013 and a Senior Research Fellow in Pathology at Melbourne University in 2003. In 2009 she was appointed a Group Leader at CSIRO and an OCE Science Leader in 2014. The specific goal of her laboratory is to understand hemopoiesis and therefore begin to address the changes and their effects in hemopoietic disease. She has focused much of her independent research career establishing models to identify where HSC reside in the BM, the cellular and extracellular components in that microenvironment as well as the mechanisms through which these regulate HSC fate. She is a highly regarded member of the international HSC field. Associate Professor Nilsson is the author of 60 publications with >4600 citations, including 2 with > 500 and 17 with >100. Her contributions have led >60 invited, keynote and plenary lectures at academic institutions (national and international) and conferences (national and international). She is currently an associate editor of Experimental Hematology and the International Journal of Hematology.

Therapeutic targeting and rapid mobilisation of endosteal HSC using a small molecule integrin antagonist

The collection of haematopoietic stem cells (HSC) for bone marrow transplant relies on their mobilisation into the peripheral blood (PB). Traditionally, this has been routinely achieved using granulocyte-colony stimulating factor (G-CSF). However, G-CSF-based mobilisation requires multiple doses over a number of days, is known to alter the function of the HSC niche as well as bone formation and can cause bone pain and spleen enlargement as well as other rare but life threatening complications. Recently the use of small molecules for HSC mobilisation has been explored, including the CXCR4 antagonist AMD3100 (Plerixafor/Mozobil). However, clinical mobilisation with AMD3100 is only effective in combination with G-CSF and the search for rapid, selective and G-CSF independent mobilisation regimes remains a topic of interest. Recently we have shown that inhibiting $\alpha_9\beta_1/\alpha_4\beta_1$ integrins with a small molecule antagonist (N-(Benzene-sulfonyl)-L-prolyl-L-O-(1-Pyrrolidinylcarbonyl)tyrosine; BOP) rapidly mobilises HSC with long-term multi-lineage engraftment potential. Additive augmentation of the engraftment of PB HSC was observed when BOP was co-administered with AMD3100. This combination effectively out-competed PB HSC mobilised with 4 days of G-CSF treatment in murine competitive transplant models. Subsequently, we demonstrated the enhanced mobilisation using the small molecule combination could be recapitulated in humanized NODSCIDIL2R$\gamma^{-/-}$ mice, where a significant increase in PB CD34+ stem and progenitor cells was observed after treatment with BOP and AMD3100. To assess the binding activity of this class of integrin antagonists on stem cell populations *ex vivo* and *in vivo*, we synthesized a related fluorescent analogue (R-BOP). Using R-BOP, we showed that this class of antagonists preferentially binds mouse and human HSC via intrinsically activated $\alpha_9\beta_1/\alpha_4\beta_1$ integrins within the endosteal niche; the region most closely associated with the bone/BM interface. More recently, we have applied this concept to the chemosensitisation of acute lymphoblastic leukaemia and acute myeloid leukaemia, whereby quiescent BM leukaemic cells were effectively dislodged from their protective endosteal BM microenvironment rendering them susceptible to chemotherapy. The results support the use of dual $\alpha_9\beta_1/\alpha_4\beta_1$ integrin inhibitor as an effective, rapid and transient mobilisation agent with promising clinical applications in stem cell therapies.

A/Professor Paul Ekert - Murdoch Childrens Research Institute

Associate Professor Paul Ekert is a paediatrician and is the Group Leader of Cancer Research at the Murdoch Childrens Research Institute. His group has several research interests, which include the study of the genetic lesions that drive paediatric cancers. This includes the use of RNA sequencing to identify novel translocations and other driving genomic features in paediatric cancers, with a focus on leukaemia. His laboratory also has a long track record of studying cytokine receptor signalling and the regulation of cell death pathways using Interleukin-3 signalling as a model.

The dose-dependent effects of microRNA-155 in leukemia

MicroRNAs are a class of non-coding, regulatory RNAs that control several critical cellular processes. A subset of microRNAs are dysregulated in cancer, and can act as oncogenes or tumour suppressors.

MicroRNA-155 (miR-155) has a well-established role as an oncogene in B cell lymphoma but has a more enigmatic role in acute myeloid leukemia (AML), in which there is evidence that miR-155 may promote or repress the development and progression of AML. We have used enforced expression of miR-155 in murine AML cell lines and AML models to explore the biology of miR-155 in AML.

We show that the capacity of miR-155 to promote or repress the ability of AML cells to form colonies and to proliferate is dependent on miR-155 expression levels. Enforced high expression of miR-155 in AML cell lines results in reduced proliferation and colony formation. However, critical long-term assays of cells transduced with miR-155 a selection in favour of an intermediate expression level accompanied by a restoration in clonogenic potential. *In vivo*, enforced expression of miR-155 in murine AML models showed no differences in disease onset and latency compared to controls, but resulted in an increased tumour burden. Most interestingly, RNA-Sequencing analysis demonstrated that the contrasting levels of miR-155 regulate a substantially different set of gene targets, with downstream consequences on transcription that are consistent with the contrasting effects of high and intermediate miR-155 levels. The intermediate levels of miR-155 we observe are the same as that seen in human AML, whereas the high levels of miR-155 have a completely different physiological counterpart. Our data shows that that the levels of this microRNA powerfully influences that gene targets it controls and the resultant phenotypes observed. MiR-155 expressed within a specific range does promote the disease progression of AML.

Professor Geoff Lindeman - Walter and Eliza Hall Institute

Professor Geoff Lindeman is Joint Head of the Stem Cells and Cancer Division at the Walter and Eliza Hall Institute (NHMRC Senior Principal Research Fellow). He is also a medical oncologist and Director of the Joint Peter Mac and Royal Melbourne Hospital Familial Cancer Centre in the Victorian Comprehensive Cancer Centre. He holds an honorary appointment as Professorial Fellow in the Department of Medicine, University of Melbourne. Lindeman leads the Centre for Translational Breast Cancer Research (TransBCR), enabled by a NHMRC Centre of Research Excellence grant. His laboratory is studying molecular and cellular regulators of normal mammary gland development and perturbations that give rise to hereditary and sporadic breast cancer. Current research is focused on characterizing the mammary epithelial cell hierarchy (the progression from long-lived stem cells to progenitor cells to mature epithelial cells) and delineating key cell fate specification and survival genes in the mammary gland. His group has also generated patient derived xenograft (PDX) models that are being used in 'proof-of-principle' pre-clinical studies, aimed at transferring promising drugs to the clinic.

Understanding stem cells to get abreast of breast cancer

Geoffrey J. Lindeman, Emma Nolan, Nai Yang Fu, Anne C. Rios, Bhupinder Pal, François Vaillant, Gordon K. Smyth and Jane E. Visvader

Stem Cells and Cancer Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3050

The mammary gland predominantly develops in the postnatal animal where it undergoes dramatic morphogenetic changes during the reproductive cycle. Postnatal development occurs in distinct stages, encompassing puberty, pregnancy, lactation and involution. Ductal morphogenesis in puberty results in the generation of an elaborate bilayered ductal tree comprising cells of the luminal and basal/myoepithelial lineages. During pregnancy, the number of epithelial cells increases dramatically, with the formation of alveolar luminal units that differentiate in late pregnancy. New cells are also required during normal homeostasis that accompanies each estrus cycle. Our group has an interest in elucidating how stem cells give rise to the normal mammary epithelial hierarchy and in understanding key developmental regulators, with a view to identifying perturbations that lead to breast cancer.

Several years ago we identified a mammary stem cell (MaSC)-enriched population in the basal compartment. Further work has revealed a hierarchy of progenitor cells, while lineage tracing studies have confirmed the existence of bipotent MaSCs, as well as long-lived progenitor cells that sustain the mammary tree. The role of female steroid hormones in regulating the epithelial hierarchy has also been explored. Although MaSCs lack steroid hormone receptors, they are exquisitely sensitive to hormones. Paracrine factors, including RANKL appear to be important regulators of basal stem and luminal progenitors in the mouse mammary gland (and LPs in human breast).

The luminal progenitor (LP) cell was found to have a molecular signature that closely resembles basal-like breast cancer, suggesting that LPs are a likely cell of origin for this clinically aggressive breast cancer subtype. Moreover, the LP cell population exhibited aberrant behaviour in ostensibly normal tissue from BRCA1 mutation carriers, indicating that the LP could be an important therapeutic target.

Professor Gail Risbridger - Monash University

Professor Gail Risbridger is an NHMRC Senior Principal Research Fellow, career academic and researcher who has >25 years' experience in Prostate Cancer research and Men's Health. She graduated from, and taught at Monash University, until becoming a founding member of the Monash Institute of Medical Research (MIMR) now known as the Hudson Institute of Medical Research. Currently, she heads the Prostate Cancer Research Group in the Department of Anatomy & Developmental Biology at Monash University, leading an internationally recognised research team of scientists and clinicians working on prostate cancer and Andrology related projects. She is one of Australia's leading prostate cancer researchers and pioneered the use of stem cells for recombination studies combining stem cell biology with endocrinology. She holds the positions of Deputy Dean, Special Projects, FMNHS, Research Director of Monash Partners Comprehensive Cancer Consortium (MpCCC) and Chair, Faculty Research Centres & Institutes Committee in the Faculty of Medicine Nursing & Health Sciences as well as advisory roles in Andrology Australia and the Freemason's Foundation Centre for Men's Health. Her academic and industry collaborations have built infrastructure and trained some of the workforce required to underpin the national research effort in Australian Prostate Cancer Research, including a National tissue bank with Victorian State Government informatics support. She has authored over 215 publications**,** and has received more than $23M in National and International grant funding since 2003. Her awards include an International Fulbright Senior Scholar Award, British Endocrine Society Asia-Oceania Medal and Honorary Life Member of Endocrine Society of Australia.

Stem cells and prostate cancer

Gail P Risbridger and RA Taylor

Monash University, Melbourne, Australia

Prostate cancer is the second most commonly diagnosed malignancy and a major cause of morbidity and death in Australian men; more than 21,808 cases are diagnosed annually. Most men have localised disease but for those patients with advanced disease the role of the AR remains at the forefront of treatment. However, they invariably exhibit disease progression despite castrate levels of circulating androgens, a condition commonly known as CRPC.

Although previous studies have focussed on the cell-of-origin of prostate cancer, it is our view that cancer repopulating stem/progenitor cells are therapeutic targets throughout disease progression. In this presentation we show how cells that escape androgen blockade are present in early stage disease and how they might be targeted, including via the stroma. As well, we consider the cancer repopulating cells in tumors from men with advanced disease that become therapy resistant.

Professor Kate Loveland - Monash University

Professor Kate Loveland is an NHMRC Senior Research Fellow with over 140 peer-reviewed publications. Her laboratory investigates signaling pathways that underpin mammalian testis development and spermatogenesis. She has particular expertise in activin/ TGF-β superfamily contributions to cellular development, with an emphasis on SMAD biology. These studies have defined downstream targets and delineated the outcome of perturbed activin signaling on the phenotype and behavior of specific cell types in the testis (somatic and spermatogenic cells). Her current work is focused on establishing the downstream targets of activin, including validation experiments that span different organ systems and analysis of target functions. Other pathways under active investigation are: WNT, Hedgehog and Snail-mediated. Her laboratory routinely uses mouse and human primary materials and cell lines, mouse models and a suite of histological, biochemical and molecular approaches. Expertise in measuring protein-protein interactions has been gained from work on how protein nucleocytoplasmic transport determines signaling outcomes. An extensive international network of collaborations (Germany, Japan, Denmark, USA) facilitates much of the work in her laboratory, including through exchange of personnel. She co-leads an International Research Training Group for PhD student training in the Molecular Pathologenesis of Male Reproduction, an exchange program between Monash University and Justus-Liebig University in Germany funded by the German Research Foundation. She is also Head of Postgraduate Research at the Monash Health Translational Precinct, with oversight of approximately 200 PhD students.

What influences male germline stem cells and testicular cancer?

A dynamic molecular dialogue between somatic and germ cells orchestrates tightly controlled germ cell epigenetic reprogramming, proliferation and quiescence, migration and differentiation required for male fertility. Events after sex determination and around the time of birth are particularly relevant to formation of testicular germ cell tumours (TGCTs) and spermatogonial stem cells. Several TGF-β growth factor superfamily members, including Activin A, influence key stages of germ and somatic cell development. In fetal and juvenile mice, activin A levels determine Sertoli and germ cell proliferation and maturation. Receptor expression profiles indicate Sertoli and germ cells are each potential targets of activin and TGFβ ligands in normal and tumourigenic adult human testes. Exposure of the TCam-2 human seminoma cell line to activin A, BMP4 and retinoic acid affected transcripts encoding activin receptors and Kit, and altered cell survival or proliferation. Exposure of testis tissue fragments from testicular cancer patients to activin A (48 hr; 50 ng/ml) reduced KIT mRNA and proteins, reinforcing the importance of regulated activin A bioactivity for testis function. This frames an understanding of how TGFβ superfamily components implicated in testicular germ cell tumours, including inhibinα, NODAL, TGFBR3/betaglycan and BMP7, may integrate to control tumor aetiology. Importantly, Activin levels rise in the fetal testis, when the genome of the earliest sperm precursors (gonocytes) is globally demethylated and vulnerable to inappropriate retrotransposon activation. This critical period for adult fertility and genetic stability features synthesis of Piwi-interacting RNAs (piRNA) and associated machinery that collectively repress transposable elements and thus protect the unmethylated gonocyte genome. We postulated that rising Activin levels in the fetal testis affect piRNA machinery production. TCam-2 cell culture with activin A (5ng/ml) influenced transcripts encoding the piRNA machinery. Targets included DNMT3l, which facilitates protective *de novo* methylation on germline DNA, and TRDR proteins, which mediate piRNA actions. We propose activin A levels influence genetic stability in the male germline by affecting piRNA activity during spermatogenesis.

Posters

1. Targeting liver cancer stem cells using aptamers against key cancer stem cell markers

Gang Zhou, George Wilson, Guang Wu, Shu Liu, Jacob George, Christopher Liddle, Liang Qiao

Storr Liver Centre, Westmead Institute for Medical Research, University of Sydney and Westmead Hospital, Westmead, NSW 2145, Australia

2. Jagged2-dependent Notch2 signaling is required for the maintenance of liver cancer stem cells

Guang Wu, George Wilson, Jacob George, Liang Qiao

Storr Liver Centre, Westmead Institute for Medical Research, University of Sydney and Westmead Hospital, Westmead, NSW 2145, Australia

3. Human model to identify genetic drivers for leukaemia in Down's Syndrome children

Melinda Tursky[1], Tim Molloy[1], To Ha Loi[1], Dmitry Ovchinnikov[2], Helen Tao[1], Ernst Wolvetang[2], David Ma[1]

[1]Blood, Stem Cell and Cancer Research Group, St Vincent's Centre for Applied Medical Research, Sydney, NSW Australia and St Vincent's Clinical School, Faculty of Medicine, Sydney, UNSW Australia

[2]Stem Cell Engineering Group, Australian Institute for Bioengineering and Nanotechnology, St Lucia, QLD Australia

4. Identification of a key stemness determinant in acute myeloid leukaemia

Florida Voli, Hangyu Yi, Jenny Y Wang

Cancer and Stem Cell Biology Group, Children´s Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW 2052, Australia

5. Targeting the self-renewal pathway of leukaemic stem cells in acute myeloid leukaemia

Hangyu Yi[1], Jennifer R. Lynch[1], Jenny Y. Wang[1,2]

[1]Cancer and Stem Cell Biology Group, Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW 2052, Australia

[2]Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia

6. Haematopoietic stem cells to generate chimeric antigen receptor (CAR)-modified T cells

Sylvie Shen[1, 2], Ning Xu[1, 2], Tracey O'Brien[1, 2] and Alla Dolnikov[1, 2]

[1]Children's Cancer Institute Australia,

[2]Sydney Children's Hospital

7. Characterising cell populations in liposarcoma

Regina Ryan[1], Vashe Chandrakanthan[1], Avani Yeola[1], David Kang[1], Trent Davidson[3], David Goldstein[1,2], Philip J. Crowe[1,2], Jia-Lin Yang[1] and Jason W. H. Wong[1]

[1]Faculty of Medicine, Prince of Wales Clinical School, University of New South Wales, Randwick NSW

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