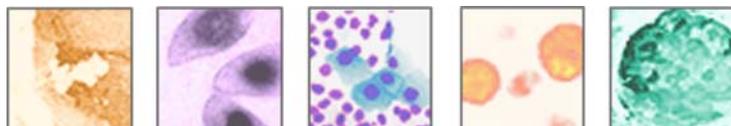


12th Stem Cell Workshop

Induced Pluripotent Stem Cells

Tuesday 4 August, 2009
10:00am to 3:00pm
Medical Foundation Auditorium
University of Sydney



Welcome to the 12th Stem Cell Workshop

The idea that all the therapeutic benefits promised by embryonic stem cells could be provided by such an accessible and uncontroversial source of cells such as skin cells has naturally excited a great deal of interest.

In the short time since human somatic cells were first induced to a pluripotent state with the potential to become virtually any cell type in the body, many important similarities to human embryonic stem cells have been established. The degree of difference between induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) and whether it has any significant consequences is an important area of investigation. Dr Andrew Laslett's laboratory at the Australian Stem Cell Centre is working on characterising iPSCs and Mirabelle Ho will talk about some of the distinctions which their research has uncovered.

In order for iPSCs to be viable for clinical use there are a number of issues that still need to be resolved regarding the methods of generating them. Dr Paul Verma, whose laboratory at the Monash Medical Research Institute is at the leading edge of this research will discuss the various merits and problems with alternative methods, as well as some of the logistical and regulatory issues. Dr Anna Michalska, who is investigating non-viral generation of iPSCs at the Monash Immunology and Stem Cell Laboratories will give an overview of approaches which avoid the clinically problematic use of viruses to induce pluripotent stem cells. Towards realising the potential of iPSCs, Prof David Kaye of the Baker IDI Heart and Diabetes Institute will discuss the challenges that still remain in the differentiation of pluripotent stem cells into heart cells as well as their delivery and integration into the myocardium. His group has recently developed a pre-clinical novel method for cell delivery in the large animal heart which he will report on..

The national "Reprogramming and Induction of Pluripotency Program" is an initiative funded by the Australian Stem Cell Centre which brings together eight Australian stem cell laboratories to address the various challenges of iPSC research in a collaborative way. The program's director, A/Prof Ernst Wolvetang will outline the strategies and report on the progress of the Brisbane node which he leads.

Before iPSCs see a successful translation to the clinic there are a number of legal and ethical considerations that require elaboration and deliberation. Although iPSCs potentially avoid ethical dilemmas associated with ESCs, there are overlapping as well as specific legal and ethical challenges to be addressed. Prof Belinda Bennet from the Faculty of Law at Sydney University will discuss issues arising from the use of iPSCs with regards to informed consent, ownership and benefit sharing with tissue donors. Important ethical and legal implications of the reproductive potential of iPSCs will be explored by Prof Isabel Karpin from the Faculty of Law at the University of Technology, Sydney, as well as the current lack of legal provisions covering this territory in Australia. Taking the promise of iPSCs to their conclusion, Dr Olivia Harvey from the University of NSW asks to what extent the use of iPSCs instead of ESCs increases the potential for the prospective development of clinical products. Dr Harvey explores the question of what a commercial model of iPSC based therapies might look like.

Enjoy this Workshop and we look forward to keeping in touch through the Network.

Kind regards,



Nola Camden
Manager



Prof Bernie Tuch
Director

NSW Stem Cell Network

WORKSHOP PROGRAM

9:30am	Registration opens / coffee
10:00am	Keynote speech Dr Paul Brock AM, Director of Learning and Development Research, NSW Department of Education / Adjunct Professor, Faculty of Education and Social Work, University of Sydney / Vice Patron, Motor Neurone Disease Association NSW
Session 1: The science of induced pluripotent stem cells in Australia	
Chair:	A/Prof Chris O'Neill, Kolling Institute of Medical Research, Royal North Shore Hospital
10:10am	Generating clinically relevant induced pluripotent stem cells Dr Paul Verma, Monash Institute of Medical Research
10:35am	Are human embryonic stem cells and induced pluripotent stem cells equivalent and does it matter? Mirabelle Ho, Australian Stem Cell Centre
11:00am	Non-viral generation of induced pluripotent stem cells Dr Anna Michalska, Monash Immunology & Stem Cell Laboratories
11:25am	Cardiovascular applications of induced pluripotent stem cells Prof David Kaye, Baker IDI Heart and Diabetes Institute
11:50am	The national reprogramming and induction of pluripotency initiative A/Prof Ernst Wolvtang, University of Queensland
12:15pm	Lunch / networking
Session 2: Community aspects of induced pluripotent stem cells	
Chair:	A/Prof Bernadette Tobin, Plunkett Centre for Ethics, St Vincent's Hospital / Aust. Catholic University
1:15pm	Who owns the stem cell? Legal and ethical issues in regulating iPS cells Prof Belinda Bennet, Centre for Health Governance, Law & Ethics, Sydney University
1:40pm	iPS cells and the legal regulation of reproduction – do we need to consider the two together? Prof Isabel Karpin, University of Technology
2:05pm	What would a commercial model look like for iPS cell based therapies? Dr Olivia Harvey, University of NSW
2:30pm	Summary and future directions Dr Bernie Tuch, Director of the NSW Stem Cell Network
2:35pm	Refreshments / networking

GENERATING CLINICALLY RELEVANT INDUCED PLURIPOTENT STEM CELLS

The demonstration that proviral expression of genes involved in embryogenesis and early development can induce mouse or human somatic cells to de-differentiate to a state indistinguishable from embryonic stem cells (ESCs) has revolutionized the generation of autologous pluripotent cells. Called induced pluripotent stem cells (iPSCs), in mice the cells have the ability to contribute to all the cells of the resulting chimera including the germ-line when injected into blastocysts. In humans the induction of pluripotency is very inefficient and the potential of insertional mutagenesis limits the use of iPSCs in a clinical setting. Approaches being explored to generate iPSCs efficiently and without genetic modification will be presented. Finally, logistical and regulatory issues to enable the translation of this extraordinary research into therapeutic outcomes will be discussed.



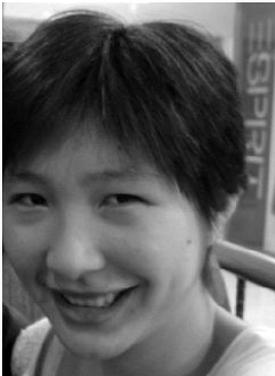
Paul J. Verma, PhD
Cell Reprogramming Laboratory, Monash Institute of Medical Research

Paul obtained his PhD at Adelaide University and subsequently joined BresaGen Ltd, Adelaide in 1995, working on transgenesis and nuclear transfer aimed at developing pigs with organs suitable for xenotransplantation. He later joined the Luminis-BresaGen Cell Therapy Program, where he established a research program aimed at providing alternatives to therapeutic cloning.

In 2001 he moved to the Monash Institute of Medical Research, where he currently heads the stem cell program. His research aims to produce autologous (patient-specific) stem cells, using cell-reprogramming techniques such as somatic cell nuclear transfer and iPS cell technology that are clinically relevant and transplantable. He has edited a book on transgenesis, cloning and stem cells, and is principal inventor on eight granted and provisional patents in the field of cell reprogramming and stem cells. He serves on the NHMRCs, Cellular Therapies Advisory Committee (CTAC), on the editorial board of Animal Reproduction Science and is a visiting Professor at the National Centre for Biological Sciences, Bangalore, India.

ARE HUMAN EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS EQUIVALENT AND DOES IT MATTER?

Support for the clinical use of induced pluripotent stem cells (iPSCs) stems from the observation that they share a high degree of similarity to human embryonic stem cells (hESCs) with regards to morphology, molecular and epigenetic profiles as well as functional properties, at least when heterogeneous populations of hESCs and iPSCs are compared. Using a novel immunotranscriptional approach we fractionated heterogeneous population of hESCs and iPSCs to enriched subfractions expressing varying levels of stemness markers TG30 and GCTM-2. These subfractions were arbitrarily termed (GCTM-2Neg-TG30Neg) P4 - (GCTM-2Hi-TG30Hi) P7. Using colony-forming assays, we demonstrate for the first time that upon fractionation, hESCs and iPSCs possess distinct in vitro and in vivo functional properties. Within 7 days of the initial FACs, P4-P7 iPSCs were observed to form colonies morphologically indistinguishable to conventional hESCs. In contrast, only P7 and to a lesser extent P6 hESC were observed to form colonies following 14 days in culture. Subsequent flow cytometric analysis, immunofluorescence and Q-PCR studies confirm the identity of these colonies as genuine stem cell colonies. Collectively, these results strongly argue that the biology of iPSCs remains terra incognita. As such further studies and cautious optimism need to be applied when considering the clinical translation of iPSCs.



Mirabelle Ho **Australian Stem Cell Centre and** **Department of Anatomy and Development, Monash University**

Mirabelle Ho graduated with first class honours in a Bachelor of Biomedical Science at Monash University in 2007. During her undergraduate years, she was awarded the Academic Excellence Award as well as several summer scholarships to undertake research at the John Curtin School of Medical Research, Departments of Biochemistry and Molecular Biology at Monash University, Monash Immunology and Stem Cell laboratories and the Australian Stem Cell Centre (ASCC). She is currently a recipient of an Australian Postgraduate Award, and an ASCC Post-graduate Supplementary Scholarship working in the laboratory of Dr. Andrew Laslett at the ASCC and the Department of Anatomy and Developmental Biology at Monash University in Melbourne. Her research focuses on gaining a better understanding of human embryonic stem cells (hESC) at a functional and molecular level. The latter involves the creation of a reporter cell line capable of detecting changes in pluripotency. In addition she is also interested in the comparison between hESCs and induced pluripotent stem cells which would shed light on factors involved in pluripotency and possible mechanisms by which these factors effect reprogramming. Mirabelle is co-first author on a manuscript entitled "Identification of human embryonic stem cell surface markers by combined membrane polysome translation state array analysis and immunotranscriptional profiling" that has just been accepted for publication in the journal *Stem Cells*.

NON-VIRAL GENERATION OF INDUCED PLURIPOTENT STEM CELLS

Recently, it was shown that the ectopic expression of a combination of transcription factors OCT3/4, SOX2, c-Myc, KLF4, NANOG and LIN28 is sufficient to reprogram mouse and human somatic cells to a pluripotent state. The resultant induced pluripotent stem (iPS) cells are similar to embryonic stem (ES) cells in morphology, gene expression pattern, epigenetic status and differentiation potential.

Initial strategies used to generate iPS cells were based on transduction of somatic cells with retroviral or lentiviral vectors carrying reprogramming factor transgenes, which resulted in the introduction of permanent genetic modifications in the host cell. This approach poses a great safety concern; not only random integration of transgenes could disrupt endogenous genes, resulting in insertional mutagenesis, but the reactivation of potentially harmful oncogenes (in particular the transgene encoding c-Myc) is likely to increase the risk of tumor formation. In addition, leaky expression of reprogramming factor transgenes can suppress differentiation process in iPS cells resulting in teratoma formation when transplanted to patients.

Successful translation of the iPS cell technology to human regenerative medicine will require identification and the development of alternative reprogramming methods. Some progress has been made by demonstrating that iPS cells can be generated, albeit at very low efficiency, without viral integration using non-integrating adenoviral or episomal vectors, transient transfection of reprogramming plasmids, transposition system and Cre-excisable viruses.

More recently, iPS cells have been generated by direct delivery of reprogramming proteins to target cells demonstrating, in principle, that a potentially safe method of deriving patient-specific cells is feasible. However, before the protein-based iPS (piPS) cell technology is brought closer to a clinical setting, it needs further optimization to achieve increased efficiency of reprogramming and stringent testing and characterization of piPS cells.

In this presentation I will attempt to critically overview approaches for non-viral generation of induced pluripotent stem cells.



Anna Michalska, PhD
Monash Immunology and Stem Cell Laboratories,
Monash University

Dr Michalska is a senior research fellow at the Monash Immunology & Stem Cell Laboratories, Monash University. The main focus of Dr Michalska's research is on the optimization of the culture conditions for the maintenance of human ES cells in pluripotent state, the derivation of patient specific hESC lines and, recently, on the study of human amnion-derived epithelial cells as a potential source of stem cells for regenerative medicine. Dr Michalska's current interests include investigating safe methods of generating human induced pluripotent stem cells that can be used for therapeutic applications. She and her colleagues at MISCL were awarded an ARC grant to pursue this research.

CARDIOVASCULAR APPLICATIONS OF INDUCED PLURIPOTENT STEM CELLS

The clear recognition that end-stage heart failure has few treatment options, has provided a strong impetus to investigate the potential therapeutic application of both embryonic and induced pluripotent stem (iPS) cells, and their capability of differentiation into cardiomyocytes. Despite this potential, many challenges remain including the optimization of differentiation in cardiomyocytes, their delivery and integration into the myocardium. Our group has been particularly active in the areas of cell delivery and we have recently developed a pre-clinical novel method for cell delivery in the large animal heart. In conjunction with their therapeutic role, iPS cells derived from specific patients also have the potential for the individualized therapy and more broadly for drug development



Prof David M Kaye, MBBS, PhD, FRACP, FACC
Baker IDI Heart and Diabetes Institute / Alfred Hospital, Melbourne

Professor David Kaye is Associate Director and Head of the Cardiology and Therapeutics Division at the Baker Heart Research Institute and a Cardiologist at the Alfred Hospital, Melbourne, Australia. His principal clinical and research interest is in the area of heart failure, particularly on novel aspects of myocardial pathophysiology in the areas of nitric oxide signaling, remodeling, autonomic control and atrial fibrillation. His group has a major emphasis on translational research, with the development of non-surgical devices for gene and cell delivery to the failing heart. He has published over 160 manuscripts in peer reviewed international journals. He serves on the Editorial Board of Circulation.

THE NATIONAL REPROGRAMMING AND INDUCTION OF PLURIPOTENCY INITIATIVE

In order to foster a national collaborative effort on iPS cell technology and future clinical application of these cells the ASCC has recently funded a program that brings together eight Australian laboratories. This collaborative stream covers iPS cell generation in a limited number of nodes and in depth analysis of iPS cell behavior and their genetic and epigenetic signatures, iPS cells as disease models and pre-clinical testing of iPS and iPS cell derived cells. We will further report on our progress with iPS cell generation at the Brisbane node and the validation of six iPS cell lines (Bris 1-6) and outline our strategies for using viral and non-viral techniques as well as epigenetic modifiers to improve the efficiency of iPS cell generation.



Ernst J. Wolvetang

Stem Cell Engineering Group, Australian Institute for Bioengineering and Nanotechnology, University of Queensland

Associate Professor Wolvetang obtained his PhD from the University of Amsterdam in the Netherlands and subsequently emigrated to Melbourne. During his time at the Monash University he worked on the regulation of apoptosis in the Department of Biochemistry and Ets-transcription factors and Down syndrome at the Monash institute for Medical research (formerly Institute of Reproduction and Development) before joining the group of Prof Martin Pera at the Australian Stem Cell Centre with the aim to apply cutting edge molecular biology techniques to human embryonic stem cells. He was one of the first to use lentiviral transduction of human embryonic stem cells (hESC) and employed this technique to stably deliver or knock-down genes in these cells and interrogate their biology. His research further lead to the discovery that the cell surface receptor CD30 is a marker for genetically unstable human embryonic stem cells, which was published in Nature Biotech (2006) and sparked an ongoing interest in the molecular mechanisms that control the genetic and epigenetic stability of hESC. At the start of 2008 Assoc Prof. Wolvetang was recruited to the Australian Institute for Bioengineering and Nanotechnology with the aim to establish human embryonic stem cell research at the University of Queensland and apply nanotechnology and bioreactor approaches to the expansion and directed differentiation of hESC. The aim is to efficiently and safely generate patient specific embryonic stem cells for regenerative therapies. In line with this goal his laboratory intends to use the expertise in viral delivery and hESC biology to expand Induced Pluripotent Cell technology in Australia. He recently became the director of the "Reprogramming and induction of pluripotency program", an initiative that brings together eight laboratories from across Australia that has this same aim.

WHO OWNS THE STEM CELL? LEGAL AND ETHICAL CONSIDERATIONS IN REGULATING IPS

The use of human embryonic stem cells for research has been the subject of much debate. Differing views over the moral status of the human embryo and about the use of human embryos in research have complicated the debate over stem cell research to date. The development of induced pluripotent stem cells (iPS) potentially avoids many of the ethical dilemmas that have been at the heart of public debates about stem cells. This paper addresses the legal and ethical challenges arising from the use of iPS. Issues that will be addressed in this paper include informed consent to donation and research, ownership of and property rights in stem cells, and benefit sharing with tissue donors.



Prof Belinda Bennet, SJD
Centre for Health Governance, Law and Ethics, Faculty of Law,
University of Sydney

Belinda Bennett is Professor of Health and Medical Law in the Faculty of Law, University of Sydney. She is a member of the University's Academic Board, Chair of the Academic Board's Academic Staffing Committee, and she is an elected Fellow of the University Senate. Within the Faculty of Law Belinda is the Director of the Faculty's Centre for Health Governance, Law and Ethics. Her previous positions in the Faculty include: Pro-Dean (Teaching Programs), Associate Dean (International), Associate Dean (Postgraduate Coursework), and as the Faculty's first Director of Teaching Development. Belinda was a founding Board Member of the Australian Institute for Health Law and Ethics (AIHLE). She is the Deputy Editor of the Journal of Law and Medicine and a member of the International Editorial Board of the International Journal of Law in Context. Belinda's research includes legal issues relating to preimplantation genetic diagnosis; an Australian Research Council-funded project (2006-08) with Wendy Rogers and Isabel Karpin on gender inequities in health research; and a current (2009-11) Australian Research Council project with Terry Carney on legal and ethical preparedness for pandemic influenza. She has published widely on issues relating to health law and ethics. Her book, *Health Law's Kaleidoscope: Health Law Rights in a Global Age* (Ashgate 2008) brings together analysis of a range of issues including regulation of reproductive technologies, legal and ethical aspects of preimplantation genetic diagnosis, health tourism, and pandemic influenza to develop deeper and innovative understandings of the changing nature of rights and autonomy, of the relationship between individual and societal interests, and of the balance between national and international regulation in contemporary society.

INDUCED PLURIPOTENT STEM CELLS AND THE LEGAL REGULATION OF REPRODUCTION - DO WE NEED TO CONSIDER THE TWO TOGETHER?

Induced pluripotent stem cells (iPSCs) have been claimed as an ethical means to generate pluripotent cells that will differentiate into specific cell types. Obviating the need for embryo destruction the creation of pluripotent cells from adult somatic cells has been hailed as a breakthrough of legal and ethical significance. Putting aside the existing limitations of the iPSC technology which, because it is still in its infancy, are unlikely to persist for long, it is important from a legal perspective to examine how the development of this technology may impact on the regulation of reproduction. There are two potential sources of concern that arise in the legal context from the development of iPSC technology. The first is the possible use of iPSCs to create embryos. When news of the breakthrough in the initial development of iPSCs was circulated in the press some scientists were quick to suggest the significant ramifications. Professor Bob Williamson, for instance, was quoted as saying "if every cell in the body has the potential to become an embryo, do people who are opposed to embryonic stem cell research believe that every skin cell deserves the respect that is accorded to an embryo made in the usual way?" While this kind of suggestion may seem extreme, the recent report of mouse pups produced from iPSCs that were used to induce development of a development-incompetent tetraploid embryo suggests that some legal consideration of the reproductive potential of the technology may need to be undertaken. Legal limits on the reproduction of human embryo clones by somatic cell nuclear transfer (SCNT) for instance, may need to be expanded to incorporate a prohibition of the creation of human embryos for reproduction by iPSC. A second potential use for iPSCs is in the development of artificial gametes. While this has the significant advantage of allowing research on egg development without the necessity for harvesting eggs from women, there is concern raised about the possible use of such artificial gametes in reproduction. While sperm have been successfully generated from human embryonic stem cells (hESCs) in the UK they have not yet used iPSC technology to do the same. Nevertheless in the UK the use of artificial gametes in reproduction is prohibited and it may be that we should consider whether such a prohibition should be introduced in Australia and be expanded to include artificial gametes generated from iPSCs. This paper will canvass these issues from a legal and ethical perspective.

1. Soren Holm "Time to reconsider stem cell ethics – the importance of induced pluripotent stem cells" *J Med Ethics* February 2008 Vol 34 No .2
2. Qtd. By Danielle Cronin, "Cell research creates new ethical concerns," *Canberra Times*, 22nd Nov 2007.
3. Xiao-yang Zhao, Wei Li, Zhuo Lv, et al "iPS cells produce viable mice through tetraploid complementation" *Nature Advance Online Publication* 23 July 2009



Prof Isabel Karpin, JSD
University of Technology, Sydney

Isabel Karpin (BA/LLB Syd; LLM Harv; JSD Col) is a Professor in the Faculty of Law at the University of Technology, Sydney. She teaches and researches in the areas of Feminist Legal Theory, Health Law, Bioethics, Reproduction and the law, Law and Culture and Constitutional Law. Her research focus has been laws that can broadly be described as regulating bodies and women's bodies in particular. This includes laws governing reproductive technologies, biotechnology and genetic technologies and the challenges these pose to legal understandings of normality, disability, individuality, and family. She is currently involved in several major research projects in the areas of reproductive technology, disability and emergent genetic technologies including two Australian Research Council Grants: The Legal Function of "Serious Disability" in Prenatal and Neonatal Health Care Settings and Enhancing Reproductive Opportunity in Australia: Reconsidering Consent, Altruism and the Legal Status of Embryos in ART Processes. She is the author and co-author of a number of articles and book chapters including most recently "The meaning of "serious disability" in the legal regulation of prenatal and neonatal decision-making" (2008) 16 *Journal of Law and Medicine* 233-245 (with K Savell); "The Uncanny Embryos: Legal Limits to the Human and Reproduction without Women" (2006) 28(4) *Sydney Law Review*; and "Genetics and the Legal Conception of Self?" in Roxanne Mykitiuk and Margrit Shildrick (eds), *Ethics of the Body: Postconventional Challenges*, (MIT Press, 2005). She is currently finalising a manuscript *Perfecting Pregnancy: Law, Disability and the Future of Reproduction* (with K Savell) to be published by Cambridge University Press in 2009.

WHAT WOULD A COMMERCIAL MODEL LOOK LIKE FOR IPS CELL BASED THERAPIES

Whilst the usefulness of adult stem cell therapies has long been established in biomedicine, therapies based on pluripotent stem cells are much more recent. Billed for over a decade now as potentially providing revolutionary new treatment protocols in biomedicine, the translation of pluripotent stem cell therapies into clinical products has faced political, cultural and financial barriers that are a mixture of ethical, legal, technical and economic issues. The recent development of induced pluripotent stem cells is widely seen to go part-way towards overcoming at least some of these barriers. But to what extent does the use of iPS cells instead of pluripotent stem cells derived from human embryos increase the potential for the prospective development of clinical products? In particular, how can using iPS cells overcome some of the currently existing financial barriers to delivering pluripotent stem cell science to the clinic? What would a commercial model for iPS cell based therapies look like?



Olivia Harvey, PhD
Faculty of Arts and Social Sciences School of History and
Philosophy, University of New South Wales

Olivia is a sociologist specialising in science and technology studies, lately focused on the sociology of stem cells. Olivia has a PhD and a BA (Hons 1) in sociology and science and technology studies from UNSW. In 2007 and 2008, Olivia was in the United Kingdom on an ESRC funded postdoctoral fellowship on 'Government strategies and commercial models: the politics of the global stem cell bioeconomy' with the Global Biopolitics Research Group at the University of East Anglia, Norwich and the Centre for Biomedicine and Society at King's College, London. Olivia was also a research fellow on the team's 'The global politics of hESC science' project for three months, contributing data on the EU and the US. Recently returned to Australia, Olivia has just started a new three-year project at the University of New South Wales on translation and innovation in Australian stem cell science. Formal participation in this project will in due course be sought from the Australian stem cell community however initial expressions of interest are welcome and can be directed to Olivia on o.harvey@unsw.edu.au, 9385 3768 or in person.



NSW Stem Cell Network

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