

2nd Annual "Induced Pluripotent Stem Cells: Production and Utility in Regenerative Medicine"

The Penridge Suite, 470 Bowes Road, London N11 1NL

Thursday, 06 October 2011

Human induced pluripotent stem cells (hiPSCs) generated by reprogramming somatic cells represent a unique opportunity for regenerative medicine. Indeed, hiPSCs can proliferate indefinitely in vitro while maintaining the capacity to differentiate into broad number of cell type. Therefore, hiPSCs could be used to produce an infinite quantity of cell type with a clinical interest. In addition, hiPSCs could enable the production of patient specific cell types which are fully immuno-compatible with the original donor thereby avoiding the need for immune suppressive treatment during cell based therapy. However, recent reports have suggested that epigenetic and genetic anomalies associated with direct reprogramming technology could limit the interest of hiPSCs for in vivo use.

This meeting will review the drawback and advantages of hiPSCs for diverse type of clinical applications

Meeting chair: *Dr Ludovic Vallier*, Anne McLaren Laboratory for Regenerative Medicine, University of Cambridge

This event has CPD accreditation and will have a discussion panel session.

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

- 9:00 – 9:45 **Registration**
- 9:45 – 10:00 **Introduction by the Chair:** *Dr Ludovic Vallier*, Anne McLaren Laboratory for Regenerative Medicine, University of Cambridge
- 10:00 – 10:30 **Liver and pancreas: regenerative medicine bench to bedside**
Professor Neil Hanley, Endocrinology & Diabetes, University of Manchester, UK
This talk will discuss the differentiation of pluripotent stem cells towards liver and pancreatic phenotypes and the potential clinical benefit that can be derived from these cells.
- 10:30 – 11:00 **Switching on pluripotency with Nanog**
Dr Jose Silva, Wellcome Trust Centre for Stem Cell Research, Cambridge, UK
Induced pluripotency requires the expression of reprogramming factors and culture conditions that support self-renewal of embryonic stem cells. The molecular mechanisms of reprogramming, including the role of endogenous transcriptional regulators, are poorly understood. Using a loss-of-function approach, we showed that the homeodomain-containing transcription factor Nanog is dispensable for the generation of reprogramming cell intermediates, but strictly required for the establishment of naive pluripotency. However, Nanog is poorly conserved in mammals and it is unknown whether naive pluripotency is unique to rodent species. We investigated whether this capacity is unique to rodent Nanog and will discuss the implications of this work.
- 11:00 – 11:05 **Speakers' photo**
- 11:05 – 11:20 **Mid-morning break and Poster Viewing**
- 11:20 – 11:50 **A novel and efficient method for high-throughput generation of karyotypically stable induced pluripotent stem cells using peripheral blood derived progenitor cells**
Dr Amer Rana, University of Cambridge Addenbrooke's Hospital, Cambridge, UK
To progress iPSC technology to the translational phase we need to improve (i) the practicalities of patient specific tissue/cellular reprogramming substrate sampling and banking, (ii) the reprogramming efficiencies associated with iPSC generation and (iii) the genomic integrity of the iPSCs generated to match to the patients 'normal' genome. I will present data introducing a new peripheral blood derived reprogramming substrate which is (i) obtainable from almost any patient, without blood mobilisation, (ii) exhibits efficiencies and kinetics of reprogramming suitable for the high-throughput generation of iPSC and (iii) can be used to generate iPSCs relatively free from genomic rearrangements.
- 11:50 – 12:20 **Translating Research Into Viable Clinical Treatments. How to build on 60 years of patient focused clinical delivery.**
Dr Simon Ellison, Commercial Manager, NHSBT Clinical Translation Partnerships, UK
- The criticality of managing the process from consent to patient.
 - Utilising open innovation partnerships can deliver treatments and revenue.
 - How to generate patient focused manufacturing and scale up.
 - Accessing validated national cold supply chains

- 12:20 – 12:50 **Epigenetic reprogramming of brain cancer stem cells**
Dr Steven Pollard, UCL Cancer Institute, London, UK
Cancer cells are driven by both genetic and epigenetic changes, but their relative contribution in driving the malignant phenotype remains unclear. We have used induced pluripotent stem (iPS) methodology to demonstrate that highly malignant and aneuploid human glioblastoma cells can be epigenetically reprogrammed. Glioblastoma-iPS cells (GiPSCs) activate expression of early embryonic markers such as NANOG, and display widespread reconfiguration of DNA methylation patterns including reactivation of aberrantly silenced tumour suppressor genes such as CDKN1C (p57KIP2). Removal of epigenetic restrictions enables these GiPSCs to enter alternative differentiation programs in vitro and in vivo. GiPSCs now provide a tractable model system to explore whether transcriptional resetting and epigenetic reprogramming in human cancer can restore normal cellular behaviour to malignant cells.
- 12:50–13:40 **Lunch and Poster Viewing**
- 13:40 – 14:30 **Question and Answer Session**
Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day
- 14:30– 15:00 **Deriving Functional Hepatocyte Like Cells from Induced Pluripotent Stem Cells**
Dr David C. Hay, MRC Centre for Regenerative Medicine, Edinburgh
With the advent of induced pluripotent stem cell (iPSC) technology, it is now feasible to generate iPSCs with a defined genotype or disease state. When coupled with direct differentiation to a defined lineage, such as hepatocyte like cells (HLCs), iPSCs may revolutionize the way we study human liver biology and facilitate the generation of efficient "off the shelf" models. The iPSC-derived HLCs exhibit hepatic morphology and express hepatic markers. Additionally, iPSC-derived HLC display CYP1A2, CYP2C9 and CYP3A4 metabolism, which is essential for drug and toxicology testing. Although these models are promising, iPSC-derived HLCs, like primary human hepatocytes, demonstrate limited viability and phenotypic instability (2 days) on the current state of the art tissue culture substratum, matrigel. To overcome the issues of phenotypic stability and viability we have screened a polymer library and identified a defined supporting basement matrix which supports liver function for a minimum of 15 days in vitro.
- 15:00 – 15:15 **STEMCCA Polycistronic lentiviral reprogramming vector and new ESGRO 2i from Merck Millipore: Effective tools for the generation and culture of robust iPS cells.**
Dr Rachel Craddock, Merck Millipore, France
As the field of reprogramming develops and alternative methods of reprogramming emerge, we have to look towards methods that generate the most robust induced pluripotent stem (iPS) cell lines in their similarity to embryonic stem (ES) cells. Reprogramming methods using viral vectors generate very robust iPS lines more efficiently, although some protocols can be lengthy and complicated.
Merck Millipore have proposed a new 2-step approach for the generation of iPS cells, using STEMCCA polycistronic lentiviral vector infection in serum containing medium followed by culture of pre iPS cells in new ESGRO 2i serum free feeder free medium to push pre-iPS cells towards a naïve pluripotent state.
Prolonged culture in ESGRO-2i culture can generate iPS cells with ES cell morphology. This can not be achieved with serum and feeder dependent cultures.
- 15:15 – 15:40 **Afternoon Tea/Coffee and Poster Viewing**
- 15:40 – 15:55 **Epigenetic signatures of stem cell identity**
Dr Véronique Azuara, Institute of Reproductive and Developmental Biology, Faculty of Medicine Imperial College London
At least two phases of pluripotency have been identified during early mouse development, immature and primed, and these are represented by the pluripotent progenitors in the inner cell mass (ICM) of blastocysts and post-implantation epiblasts, respectively. Here we show that human embryonic stem (hES) cells can reversibly adopt distinct epigenetic states underlying immature versus epiblast-like identities, as delineated at the DNA replication timing level. We provide direct evidence that SMAD2/3-associated histone acetyltransferase p300 activity can impinge on this balance with high levels of histone acetylation at key developmental genes favouring a stable epigenetic state in ICM-like hES cell populations. Importantly, this is accompanied by fluctuations in the expression of NANOG and REX1 and a change in hES cell's functional properties, as judged by their responsiveness to differentiation-inducing signals and capacity to reprogram mouse B-lymphocytes in cell fusion experiments. Taken together, these findings strongly support the notion that pluripotency is not a fixed state and reveal how modulations in p300-mediated histone acetylation levels might instigate dynamic epigenetic and functional heterogeneity within hES cell cultures.

15:55 - 16:25 **Development of robust, scalable, and synthetic systems for the maintenance of pluripotency and subsequent differentiation**

Dr Scott McRae, Cell Guidance Systems Ltd, Cambridge, UK

Objective: In driving hES cell technology towards widespread application considerable effort has been focused on the improvement of culture conditions and on enabling efficient differentiation. We have established two technologies which will better enable researchers to achieve these aims. *Methods:* The use of feeder based protocols for the creation, expansion and banking of hES cell lines are well established. The advances in technology for feeder free culture have predominantly relied on the cultivation of pluripotent cells in colony based systems, commonly used in conjunction with ill-defined matrices. We have developed a process to allow enzymatic single cell passaging in a medium in which Wnt3a and bFGF are interchangeable by a method utilising small molecule control of beta-catenin interaction with its binding partners in concert with additional growth factor supplementation. This medium can be partnered with chemically defined or synthetic matrix components to provide exemplary consistency of phenotype within and between passages. Following extended passage the cells retain the undifferentiated phenotype as evidenced by expression of markers of the undifferentiated state and capacity for in-vitro differentiation into the three germ lineages. In addition we have formulated a range of growth factors with enhanced efficacy at a level of multiple orders of magnitude when comparing equivalent molar concentrations through multivalent conjugation to carrier substrates. *Conclusion:* The development of improved culture systems for pluripotent cells and the increased efficacy of growth factors in inducing and establishing differentiated progeny are essential requirements if stem cell technology is to fulfil its potential and overcome technical and economic barriers. Taken together these two advancements have the potential to transform stem cell research and the development of cellular therapeutics.

16: 25 – 16:55 **Biobanking iPSC Lines**

Dr Glyn Stacey, UK Stem Cell Bank, Division of Cell Biology and Imaging, UK

The UK Stem Cell Bank was established in 2003 and distributes ethically sourced pluripotent stem cell lines. In addition to supplying cell for research the bank is also licensed to bank, test and distribute cells for human application and has helped to support the regulation of stem cell therapy in the UK. The advent of human iPSC technology in 2007 brought exciting new opportunities for the generation of bespoke genotypes of pluripotent cell lines. These are currently being explored to generate disease models *in vitro* and may in the future, find applications in cell therapy. This talk will explore the fundamental scientific and regulatory issues that researcher should be aware of to ensure that their research with iPSC lines does not become compromised or invalidated.

16:55 - 17:00 **Chairman's summing up:**

*This meeting was **organised by Euroscicon** (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry.*

Dont forget to sign up to Euroscicons' e-newsletter at www.euroscicon.com/signup.htm to keep up to date with European Life Science news and events and to be notified of the follow up to this event

Connect with us on

Nature network - <http://network.nature.com/groups/euroscicon/>



- <http://www.linkedin.com/groups?gid=1939569>



- <http://www.facebook.com/group.php?gid=70847076549>



- <http://twitter.com/Euroscicon/>

About the chair

Dr Ludovic Vallier is a member of the Department of Surgery and junior principal investigator in the newly opened Anne McLaren laboratory for regenerative medicine (LRM, Cambridge). The Vallier laboratory study mechanisms controlling differentiation of pluripotent cells pancreas and liver. These studies use human Embryonic Stem Cells and human induced pluripotent stem cells as an in vitro model of development in combination with functional analyses. Overall, the objective of the Vallier laboratory objective is not only knowing how to control differentiation of human ESCs into specific endodermal cell types (including pancreas and liver progenitors), but also to generate fully functional cell type for clinical applications. hPSCs and liver metabolic diseases.

About the Speakers

Steven Pollard studied Biochemistry as an undergraduate at the University of Bath, before carrying out his PhD in the Department of Developmental Biology at the MRC National Institute for Medical Research (NIMR). In 2002 he moved to Edinburgh to work as a postdoctoral scientist with Prof Austin Smith FRS at the Institute for Stem Cell Research (ISCR), where he investigated the conversion of embryonic stem cells to neural stem cells. In 2006 he relocated with Prof Smith to the Wellcome Trust Centre for Stem Cell Research at the University of Cambridge and was awarded a Kaye Prize Fellowship (Christ's College) and Beit Memorial Research Fellowship. He moved to the new UCL Cancer Institute in February 2010 to establish his own independent laboratory in the Samantha Dickson Brain Cancer Unit. He continues to study the molecular and cellular mechanisms that regulate neural stem self-renewal and differentiation and how these operate in the context of human brain cancer.

Véronique Azuara has worked for several years in the field of developmental biology and genetics moving from lymphocyte development (PhD training at the Pasteur Institute – Paris) to epigenetic studies (post-doctoral training at the MRC/Clinical Sciences Centre – London) to explore how chromatin shut down contributes to lineage restriction and maintenance of cell fate identity through development. Since being at the Institute of Reproductive and Developmental Biology (Imperial College London), her group has focused on understanding how potency and differentiation are critically balanced in stem cells and in the early embryo.

Rachel Craddock completed her Ph.D. in the department of Immunology at Birmingham University under the supervision of Prof. Janet Lord. Rachel then moved to Cambridge to take up a postdoctoral research position with Prof. Sabine Bahn at the Babraham Institute, devising functional cell models to study underpinning mechanisms of neuropsychiatric disorders, such as schizophrenia. She relocated to the Institute of Biotechnology, University of Cambridge, to continue this work and also to identify protein, transcript and secreted biomarkers for Schizophrenia and Bipolar disorder for the development of biosensors. Rachel then moved to Millipore to become UK and Ireland stem cell specialist, responsible for developing stem cell portfolio UK sales, for beta testing and launch of related tier 1 products and for providing technical help and advice to stem cell customers through meetings and seminars.

Jose Silva, received his first degree in Biology from the University of Porto, in Portugal. He then went on to do his PhD studies at Imperial College under the supervision of Professor Neil Brockdorff on heritable silencing mechanisms during mouse development. Following this, he moved to Professor Austin Smith's laboratory at the University of Edinburgh as an EMBO post-doctoral fellow to investigate factors involved in nuclear reprogramming. This work has led to discoveries in the field of induced pluripotency, which is his current area of research.

Glyn Stacey has a background in public health and cancer research and has worked on the development of cell substrates for manufacture of biological medicines. He is currently Head of Division of Cell Biology and Imaging and Director for the UK Stem Cell bank, a licensed clinical tissue bank, at the National Institute for Biological Standards and Control at South Mimms, UK. The work of his group covers safety and quality issues in cell therapy, cells used for manufacturing purposes, development of novel cell-based assays and the development of reference materials for tissue typing and diagnosis of genetic disorders. This work includes the need for scale up of preservation techniques and long term storage of DNA and cell lines of various types including human stem cell lines and cells used in bioassays and vaccine production. Glyn serves on numerous steering groups for organisations promoting and funding regenerative medicine and for many years as a committee member for the Society for Low Temperature Biology. He is a Professor in biotechnology and cryobiology at the University of Bedfordshire. He has also chaired the scientific advisory board for a Public Private Partnership not-for-profit company called Stem Cells for Safer Medicine.
August 2011

Dr David Hay (DH) is a RCUK Fellow and Group Leader at the University of Edinburgh's MRC Centre for Regenerative Medicine. DH has worked in the field of pluripotent stem cell biology over the last decade. His research has highlighted the important role that cell physiology and chemical biology plays in the generation of high fidelity models of human liver function from pluripotent stem cell populations. The impact of this work has led to over 20 publications in the stem cell field and regular appearances at high profile international conferences.

Simon Ellison is developing strategies that are enabling the National Blood Service to utilise its technical skills, GMP facilities, and clinical contacts to provide contract manufacturing services to the growing cellular therapy field, under the brand of Clinical Translation Partnerships (CTP). Simon has an MSc in Environmental Science from Newcastle University and subsequently an MBA from Oxford Brookes University focusing on the management of innovative collaborations. Simon's career started with Sartorius, managing both national and international commercial channels, and launching new products into emerging markets. He has since worked in a variety

of bio-pharmaceutical markets ranging from antibodies to ultra-pure water, delivering novel strategies to take companies forward. Simon now brings these commercial skills into the not-for-profit sector, initially as Commercial Director for the National Pharmacy Association, managing a partnership based turnover of £7m and developing innovative partnerships with Santander and Learn Direct. He now sits on the BIA's Cellular Therapy & RegenMed Industry Group Advisory Committee, and works within the National Blood Service driving strategies to utilise their clean rooms, skills, knowledge and logistics to help regenerative medicine companies translate their research into commercially viable treatments. Clinical Trial Partnerships (CTP) enables companies, academics and clinicians to develop their production into GMP systems, optimise the processes, and develop viable cold supply chains in partnership with the National Blood Service. This gives the cellular therapy market access to a unique skill set built within the NHS and currently delivering over 2 million cellular therapies annually.

Amer 'Moo' Rana's PhD was with Dr Rosa Beddington at NIMR, Mill Hill on body axis establishment in vertebrates. This was followed by studying germ layer induction and differentiation with Professor Jim Smith at the Gurdon Institute, Cambridge. In January 2010 he was awarded the British Heart Foundation Lectureship in Regenerative Medicine and started his lab in the Dept of Medicine, Addenbrooke's Hospital, Cambridge. His research interests are concerned with understanding the cellular and genetic mechanisms that underlie cell-fate decisions during (i) the embryogenesis, maintenance and repair of the cardiovascular and pulmonary systems and (ii) direct programming to pluripotency and cardiovascular/pulmonary fates.

Neil Hanley (FRCP, PhD) is Chair of Medicine and Wellcome Trust Senior Fellow in Clinical Science at the University of Manchester. He is a consultant endocrinologist at the Central Manchester University Hospitals NHS Foundation Trust (CMFT) where he also directs the Academy for Training & Education at the Manchester NIHR Biomedical Research Centre. He researches human developmental biology, focusing on endocrinology and aspects of stem cell biology. A major interest is how beta cells develop in the pancreas. Understanding development is relevant to cell replacement and regeneration of beta cells as novel therapy for diabetes. This includes focus on the transcription factor, SOX9, where his group collaborates with that of Dr Karen Piper Hanley. It is emerging that SOX9 plays a major role in regulating extracellular matrix components both during development and in disease such as fibrosis. He is also part of the Stem Cells for Safer Medicine consortium (www.SC4SM.org) with the ultimate goal of generating hepatocyte-like cells for drug toxicity screening. This latter work is in collaboration with colleagues at the MRC Centre in Drug Safety Science at the University of Liverpool.

Key Words: iPSC, hepatocyte, liver, CYP p450, translation, supply chain, delivery, GMP, manufacturing, drug, pluripotent stem cell, hepatocyte, pancreas, liver; beta-cell, Stable Karyotype, High-throughput efficiency, Embryonic Stem cells, Induced pluripotency, Nanog, neural stem cell, glioblastoma, DNA methylation, reprogramming, iPS cells, reprogramming, Pluripotency, STEMCCA, ESGRO 2i, Pluripotency; reprogramming; chromatin signatures; DNA replication timing; histone acetyltransferase p300

Media Sponsors



Natural Standard



Registration Web Site:

www.regonline.co.uk/stemcell2011